



Unexpected diversity and new species in the sponge-Parazoanthidae association in southern Japan[☆]



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ABSTRACT

Currently the genera *Parazoanthus* (family Parazoanthidae) and *Epizoanthus* (family Epizoanthidae) are the only sponge-associated zoantharians (Cnidaria, Anthozoa). The Parazoanthidae-sponge associations are widely distributed in tropical and subtropical waters from the intertidal to the deep sea in the Atlantic and Indo-Pacific Oceans. However, the taxonomic identification of both parties is often confused due to variable morphology and wide ecological ranges. In particular, Parazoanthidae species diversity remains poorly understood in the Indo-Pacific. In the present study, the diversity of the sponge-zoanthid association in the Indo-Pacific was investigated with 71 Parazoanthidae specimens collected from 29 different locations in Japan ($n = 22$), Australia ($n = 6$) and Florida, USA ($n = 1$). For all specimens morphological analyses were performed and total DNA was extracted and amplified for four DNA markers (COI-mtDNA, mt 16S-rDNA, ITS-rDNA and ALG11-nuDNA). The combined data demonstrate that the specimens of this study are clearly different from those of all described *Parazoanthus* species, and lead us to erect *Umimayanthus* gen. n., within family Parazoanthidae, containing the three newly described species *U. chanpuru* sp. n., *U. miyabi* sp. n., *U. nakama* sp. n. The new genus also includes the previously described species *U. parasiticus* (Duchassaing and Michelotti, 1860; comb. nov.), previously belonging to the genus *Parazoanthus*. Neighbor joining, maximum likelihood and Bayesian posterior probability phylogenetic trees clearly demonstrate the monophyly of *Umimayanthus* gen. n. to the exclusion of all outgroup sequences. The phylogenetic results were also compared to morphological features, and polyp sizes, amount of sand content in tissues, types of connections between polyps, and cnidae data, in particular holotrichs-1, were useful in distinguishing the different species within this new genus. This new genus can be distinguished from all other Zoantharia by a unique and conserved 9 bp insertion and a 14 bp deletion in the mt 16S-rDNA region. Additionally, compared to *Parazoanthus* sensu stricto (i.e. *P. axinellae* [Schmidt, 1862]), *Umimayanthus* spp. are only found associated to sponges, and have a coenenchyme much less developed than *Parazoanthus* sensu stricto. Each new species can be distinguished from other congeners by a unique DNA sequence, numbers of tentacle, maximum sizes of holotrichs, associated sponge morphology, and colony morphology. The identification of the host sponge species is the next logical step in this research as this may also aid in the distinction of *Umimayanthus* species.

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1. Introduction

Zoantharians (Cnidaria: Anthozoa) are benthic anthozoans generally characterized by forming clonal colonies, with polyps having two rows of tentacles, a single ventral siphonoglyph and unpaired

mesenteries added in couples. Zoantharia is divided in the suborders Macrocnemina and Brachyrcnemina (Haddon and Shackleton, 1891b) based on mesenterial organization, with the fifth pair of mesenteries complete and incomplete, respectively. This suborder division is supported by information on sexual reproduction, with planktonic larvae only reported for Brachyrcnemina (Ryland, 1997; Ryland et al., 2000), but molecular analyses have revealed that the suborders are not monophyletic (Sinniger et al., 2005, 2010). Currently the only sponge-associated zoanthid genera are *Parazoanthus* (family Parazoanthidae) and *Epizoanthus* (family Epizoanthidae), both within Macrocnemina.

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The type genus of the family Parazoanthidae Delage and Herouard, 1901, genus *Parazoanthus*, was established in 1891 by Haddon and Shackleton (1891a) based on the type species *P. axinellae* (Schmidt, 1862), and was defined as:

“Macrocnemic Zoantheae, with a diffuse endodermal sphincter muscle. The body-wall is incrustated. The ectoderm is continuous. Encircling sinus as well as ectodermal canals, lacunae and cell-islets in the mesogloea. Dioecious. Polyps connected by thin coenenchyme.”

[Haddon and Shackleton, 1891a]

The species-level taxonomy of the genus *Parazoanthus* has been a challenge throughout its taxonomic history, as the ecological ranges of the genus are wide and useful morphological characteristics are few (number of tentacles/septa, color, sphincter muscle shape and position, and nematocyst types, size and distribution; Sinniger et al., 2005). As well, destructive methods of examination are frequently required for analyses (Reimer et al., 2010).

To overcome these problems molecular approaches have been used to study the phylogeny of zoantharians. Sinniger et al. (2005) used mt 16S-rDNA and 12S-rDNA to produce the first basic phylogeny of the order Zoantharia; the study concluded that all genera examined were monophyletic to the exception of *Parazoanthus*, which had multiple independent and well-supported clades. Importantly, each of these clades had apparent specificity for particular substrates such as sponges and hydrozoans. Later, Sinniger et al. (2010, 2013) using a multiple DNA marker approach coupled with ecological (substrate specificity) and morphological characteristics divided the Parazoanthidae into (1) *Parazoanthus* sensu stricto living on sponges, while (2) erecting the genus *Antipathozoanthus* within Parazoanthidae for species associated with antipatharians, (3) the genus *Hydrozoanthus* within the family Hydrozoanthidae for species associated with hydrozoans, and the genera *Kulamanamana*, *Zibrowius*, *Hurlizoanthus*, *Kauluzoanthus* and *Bullagummizoanthus* associated to deep-sea octocorals. However, they noted that *Parazoanthus* was a polyphyletic group composed of three subclades, only distinguishable using genetic data. Thus genus *Parazoanthus* was systematically redefined as macrocnemic zoantharians frequently epizoic on sponges (Sinniger et al., 2010). Currently 16 species are recognized as valid (WoRMS, 2015).

The sponge-parazoanthid association is common and world-wide distributed. Historically this association has been well investigated in tropical and subtropical waters in the Western and Central Atlantic (Crocker, 1978; West, 1979; Crocker and Reiswig, 1981; Swain and Wulff, 2007; Swain, 2009, 2010; Reimer et al., 2014a), with several species described. However, few taxonomic studies have been performed in the Indo-Pacific region, and currently *P. aruensis* Pax, 1911 from Indonesia, *P. juan-fernandezii* Carlgren, 1922 from Chile, *P. elongatus* McMurrich, 1904 from New Zealand and Chile, *P. darwini* Reimer and Fujii, 2010 from the Galapagos, and *P. lividum* Cutress, 1971 from Australia are the only formally described sponge-associated *Parazoanthus* species in the Pacific Ocean. Nevertheless, various undescribed *Parazoanthus* species have been noted in recent literature (Sinniger et al., 2005, 2010; Reimer et al., 2014b and c).

This study investigates the species diversity of sponge-associated parazoanthids in the Indo-Pacific using specimens from Japan and Australia, and in particular from the Ryukyu Islands in southern Japan. Multiple molecular markers (COI-mtDNA, mt 16S-rDNA, ITS-rDNA and ALG11-nuDNA) coupled with environmental (substrate, depth) and morphological data (polyp dimensions, colony structure, polyp arrangement, cnidae analyses) were utilized, and results compared with previously reported parazoanthid species. Based on this research we erect *Umimayanthus*

gen. n. within Parazoanthidae, containing three new species described herein. In addition, *U. parasiticus*, (Duchassaing & Michelotti, 1860) (comb. nov) is moved into this new genus from *Parazoanthus*.

2. Materials and methods

2.1. Specimen collection

Specimens were collected from 29 different locations in Japan ($n = 22$), Australia ($n = 6$) and USA Florida ($n = 1$) (Fig. 1). Specimen collection was done by SCUBA between 1.5 to 30 m depth. In total 71 specimens were examined in this study (Table A.1). For most specimens, photographic images and environmental data (location, depth, and habitat) were recorded, and samples from larger colonies were extracted using a diving knife and sealable plastic bags; the size of the specimens was proportional to the size of the colony in order to leave an *in vivo* reference for posterior analyses, as far as possible. All specimens were preserved in 95–99% ethanol and specimen field information was organized into a digital database. The specimens were deposited into the Marine Invertebrate Systematics and Ecology (MISE) collection at the University of the Ryukyus, Okinawa, Japan unless otherwise noted for type specimens.

2.2. DNA extraction, PCR amplification and sequencing

Total DNA was extracted from ethanol-preserved samples using the guanidine extraction protocol as in Sinniger et al. (2010). The extracted DNA was then amplified for COI-mtDNA (mitochondrial cytochrome oxidase subunit I), mt 16S-rDNA (mitochondrial 16S ribosomal DNA), ITS-rDNA (nuclear internal transcribed spacer region of ribosomal DNA), and ALG11-nuDNA (asparagine-linked glycosylation 11) regions using standard Taq polymerase in ReadyMix solution (Qiagen, Tokyo, Japan).

The COI-mtDNA region was amplified with the primers HC02198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'; Folmer et al., 1994) and COIzoanF (3'-TGA TAA GGT TAG AAC TTT CTG CCC CGG AAC-5'; Reimer et al., 2007a) with a PCR thermal sequence of 15 min at 95 °C followed by 35 cycles of: 30 s at 94 °C, 1 min at 40 °C, 1 min 30 s at 72 °C, with a final elongation step of 7 min at 72 °C (Fujii and Reimer, 2011). For the mt 16S-rDNA region the primers were 16Sant1a (5'-GCC ATG AGT ATA GAC GCA CA-3') and 16SbmoH (5'-CGA ACA GCC AAC CCT TGG-3'; both from Sinniger et al., 2005) with a PCR thermal sequence of 15 min at 95 °C then 40 cycles: 30 s at 94 °C, 1 min at 52 °C, 2 min at 72 °C, with a final elongation step of 5 min at 72 °C (Sinniger et al., 2005). The ITS-rDNA region was amplified using the primers Zoan-f (5'-CTT GAT CAT TTA GAG GGA GT-3') and Zoan-r (5'-CGG AGA TTT CAA ATT TGA GCT-3'; both from Reimer et al., 2007b) with a PCR thermal sequence of 15 min at 94 °C then 35 cycles: 1 min at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, with a final elongation step of 10 min at 72 °C (Reimer et al., 2007b). The ALG11-nuDNA region was amplified using two set of primers ALG11-D1 (5'-TTY CAY CCN TAY TGY AAY GCN GGN GG-3'), ALG11-R1 (5'-ATN CCR AAR TGY TCR TTC CAC AT-3'); ALG11-D2 (5'-TGY AAY GCN GGN GGN GGN GA-3'), and ALG11-R2 (5'-CCR AAR TGY TCR TTC CAC ATN GTR TG-3') in a three step nested PCR as explained in Belinky et al. (2012) with modifications. First, with primers ALG11-D1 and ALG11-R1 the following thermal sequences were used: 15 min at 95 °C then 13 cycles: 1 min at 95 °C, 1 min per 1 °C touchdown from 52 °C to 40 °C and 2 min at 72 °C, then 20 cycles: 1 min at 95 °C, 1 min at 52 °C and 2 min at 72 °C, with a final elongation of 10 min at 72 °C. Second, with the same primers a re-amplification was done with the following thermal sequence:

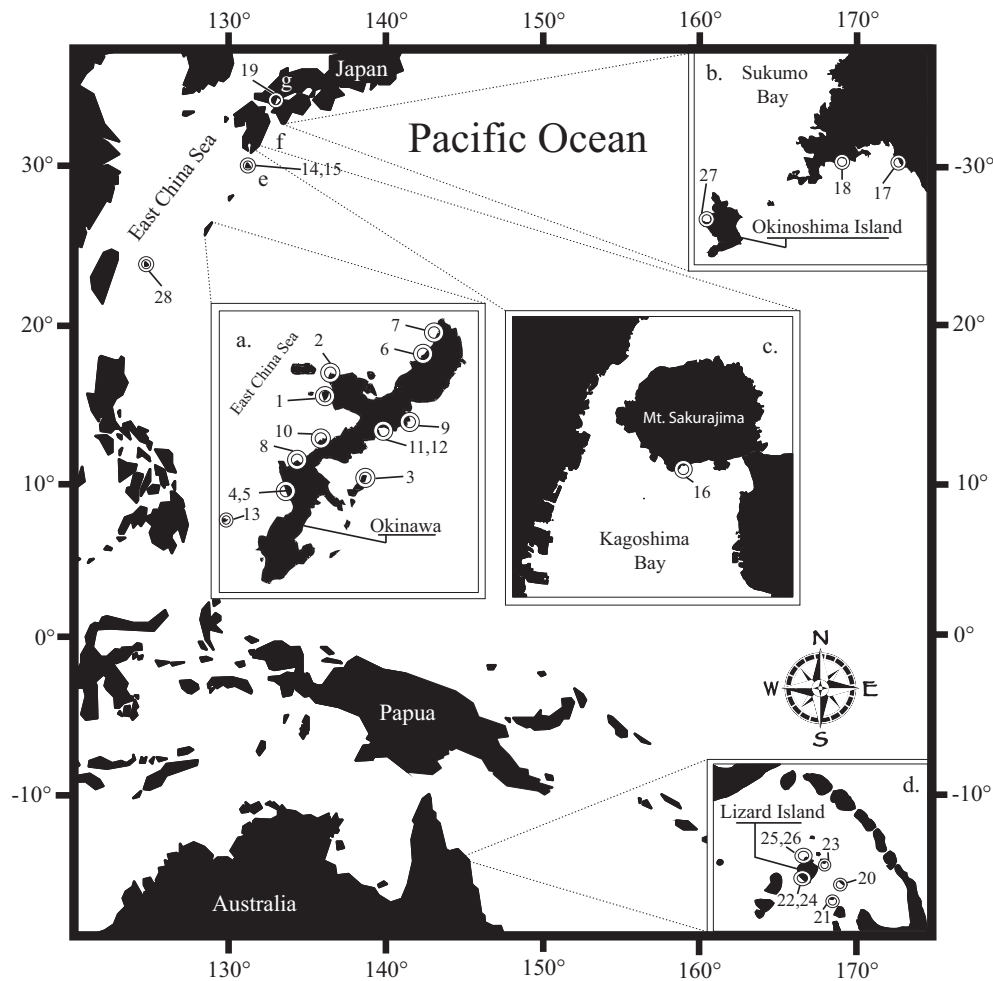


Fig. 1. Map of the western Pacific showing location of the specimens used in this study (⊙); Inset (a) Okinawa, (b) Otsuki, Kochi, (c) Sakurajima, Kagoshima, (d) Lizard Island, Queensland, (e) Yakushima, Kagoshima, (f) Ainan, Ehime, and (g) Suooshima, Yamaguchi. The numbers indicate the locations where the specific samples were collected, for the reference information see [Appendix A. Suppl. Data 1](#).

15 min at 95 °C then 35 cycles: 1 min at 95 °C, 1 min at 52 °C and 2 min at 72 °C, with a final extension of 10 min at 72 °C. All PCR products of the second step were cleaned by Shrimp Alkaline Phosphatase (SAP; TaKaRa, Japan) to eliminate short sequences and remaining primers. Third, with primer ALG11-D2 and ALG11-R2 a nested amplification was done with the following thermal sequence: 15 min at 95 °C then 35 cycles: 1 min at 95 °C, 1 min at 52 °C and 2 min at 72 °C, with a final extension of 10 min at 72 °C.

The final amplified products were checked by 1.5% agarose gel electrophoresis, and then the positive amplification bands were cleaned by SAP and sent for sequencing to external companies (Macrogen, Tokyo or Fasmac, Kanagawa).

2.3. Phylogenetic analyses

Obtained nucleotide sequences of COI-mtDNA, mt 16S-rDNA, ITS-rDNA, and ALG11-nuDNA regions were manually inspected using Se-Al v2.0a11 (Rambaut, 2002) and 4Peaks v1.6 (Griekspoor and Groothuis, 2005) to obtain consensus sequences for each sample. The consensus sequences were initially aligned using ClustalW v2.11 (Larkin et al., 2007) and manually refined. Some of the sequences used in Reimer et al. (2008), Sinniger and Häussermann (2009), Sinniger et al. (2010) and Swain (2009) were added to this study using the above methods, the terminal regions in the alignments were trimmed, and shorter or poor quality

sequences were eliminated (Appendix A. Suppl. Data 1 and 2). In this way four preliminary aligned datasets were generated; 490 sites of 92 sequences for COI-mtDNA, 605 sites of 82 sequences for mt 16S-rDNA, 900 sites of 88 sequences for ITS-rDNA, and 710 sites of 61 sequences for ALG11-nuDNA. COI-mtDNA, mt 16S-rDNA, ITS-rDNA and ALG11-nuDNA alignments were then automatically edited by GBlock v0.91 (Castresana, 2000) to eliminate ambiguities, poorly aligned positions and divergent regions; the configuration was set to allow small final blocks, gap positions between final blocks and less strict flanking positions for mt 16S-rDNA and ITS-rDNA. After GBlock editing four strict aligned datasets were obtained; 487 sites of 92 sequences for COI-mtDNA, 585 sites of 82 sequences for mt 16S-rDNA and 554 sites for 88 sequences for ITS-rDNA, while the ALG11-nuDNA alignment remained unchanged from above. A second alignment for ITS-rDNA including only sequences representatives for clades A, B and C sensu Sinniger et al. (2010) and *Umimayanthus* gen. n. was constructed and the resulting alignment consisted of 875 sites and 22 sequences. This second alignment was not edited by GBlock v0.91 (Castresana, 2000). The datasets are available upon request from the author.

Phylogenetic reconstruction was done by three methods for each region; Neighbor-joining (NJ; Saitou and Nei, 1987), Maximum-likelihood (ML; Cavalli-Sforza and Edwards, 1967) and Bayes posterior probability (BPP; Rannala and Yang, 1996). NJ trees were constructed with the program CLC Free Workbench v3.0 (Aarhus, Denmark) with the Hasegawa–Kishino–Yano genetic

distance model (HKY; Hasegawa et al., 1985) and 1000 bootstraps replicate trees were constructed using the same method. ML trees were built using PhyML v3.0 (Guindon and Gascuel, 2003) with the general time reversible model (GTR; Rodriguez et al., 1990) for nucleotide substitution, following a gamma distribution with eight categories of substitution rates and 1000 bootstrap trees. BPP trees were generated using Mr.Bayes v3.2.1 (Huelsenbeck and Ronquist, 2001) using the GTR model for nucleotide substitution, following an inverted gamma distribution with eight categories for the rate of variation, a chain length of 1,000,000 generations with a temperature of 0.2, a subsampling frequency of 200 and a burn-in length of 30,000 for all the alignments.

2.4. Morphological analyses

Cnidae were extracted by squishing individual polyps of each examined specimen against a slide glass and analyzed using a differential interface contrast microscope (Nikon Eclipse 80i). Cnidae were counted up to 500 by dividing the glass cover into eight transects separated by 2 mm. When it was not possible to reach 500 a maximum of seven transects were counted. Cnidae sizes were measure using ImageJ v1.45s (Rasband, 2012). For classification of cnidae the categories proposed by England (1991) and Ryland and Lancaster (2003) were used, but basitrichs and microbasic b-mastigophores were pooled together following Schmidt (1974), Hidaka et al. (1987), Hidaka (1992) and Fujii and Reimer (2011), and therefore six categories were used; spirocysts, b-mastigophores, p-mastigophores, basitrichs, holotrichs-1 and holotrichs-2 (large holotrichs and small holotrichs in previous works, respectively; Reimer and Sinniger, 2010; Fujii and Reimer, 2011). For each species group average cnidae and polyp sizes were computed as $\text{Avg.} = \sum(n_{i-\min} + n_{i-\max})/n$, where “ n_i ” values were the minimum and maximum measures per sample per group and “ n ” the total number of data points. The samples employed in the cnidae analyses were selected to represent each of the phylogenetic groups found in the molecular analyses.

Maximum and minimum polyp diameter ($n = 1\text{--}20$ polyps/colony, measurements intersecting when possible) and the distances between polyps per sample were measured ($n = 2\text{--}20$ polyps/colony) using a dissection microscope and the software ImageJ v1.45s (Rasband, 2012). The sand content in tissues was quantified on a qualitative scale from 0 to 3, with 0 being lowest (almost no sand present) and 3 being the highest amount of sand content (heavily incrustated) in tissue. These analyses were performed by direct inspection of preserved polyps under dissection microscope. The conditions of polyps as connected or solitary were classified into three categories: a. connected on the surface (as in *P. swiftii*), b. solitary (as in *U. parasiticus*), or c. connected under the surface (as in *Epizoanthus cutressii*); these categories followed sketches from Swain and Wulff (2007). The colors of polyps and host sponges were recorded using *in situ* images when available.

2.5. Histological analyses

For each phylogenetic group, cross-sections of polyps were made with paraffin in order to analyze mesogleal sphincter muscles. However, none of the obtained cross-sections or images (65 slides and 229 images) was usable due to sand content and/or orientation of the polyps, and we consider zoantharian polyps of this small size to be very difficult to examine by regular histological methodology.

2.6. Statistical analyses

Comparatives analyses were performed on all morphological data between monophyletic groups found in the phylogenetic

analyses. Sample sizes varied between the different phylogenetic groups. Levene's test was used to evaluate if the variances were homogeneous among the clades, and in such cases one-way ANOVA was used to compare the means across the datasets in order to detect which characteristics were significantly different among phylogenetic groups. To detect differences between groups, an independent two-sample *t*-test assuming equal variances was applied by pairs. If the variances were not homogeneous, the Kruskal–Wallis test was used to evaluate whether the data among clades followed the same distribution or not. To compare between groups, an independent two-sample *t*-test with the Cochran–Cox correction was applied by pairs. All statistical analyses were performed at 95% confidence levels.

3. Systematics

Family Parazoanthidae, Delage and Hérourard, 1901.

3.1. *Umimayanthus* gen. n

LSID: urn:lsid:zoobank.org:act:A7C6F356-9128-41EA-B108-271EC70B82FF

Type species: *Umimayanthus chanpuru* n. sp.

Etymology. Named ‘Umimaya-’ from the Okinawan dialect of Japanese word “海綿” (うみまやー) meaning ‘sponge’ as reference to the obligate association of this genus with sponges, and “anthus” the greek word for ‘flower’ often used to end anthozoan genera.

Synonymy. *Parazoanthus* Clade B sensu, Sinniger et al., 2010.

General description. *Umimayanthus* gen. n. exclusively associated with sponges, usually encrusting and cushion sponges, occasionally with massive sponges. Polyps generally scattered over the sponge surface, but can form defined stoloniferous chains in lines, or form groups of two to three connected polyps. Polyps may be solitary or connected to each other by a stolon through a thin but clearly visible coenenchyme either over or under the sponge surface. Polyps with sand particles and detritus incrustated in column. Tentacles equal or longer than the expanded oral disc diameter.

Umimayanthus gen. n. currently includes four species, *U. chanpuru* sp. n., *U. miyabi* sp. n., *U. nakama* sp. n., as well as the previously described species *U. parasiticus* (comb. nov.), which was previously placed within the genus *Parazoanthus*.

Diagnosis. *Umimayanthus* gen. n. can be distinguished from all zoantharians including *Parazoanthus* spp. by a highly conservative and unique insertion of 9 bp in length (from position 556 to 564 in alignment) and one deletion of 14 bp long (from position 574 to 587) in the mt 16S-rDNA region (Fig. 2).

Remarks: *Umimayanthus* spp. can be confused with *P. puertoricense* West, 1979 and other “*Parazoanthus*” grouped in “clade C” sensu Sinniger et al. (2010), which are morphologically similar but genetically clearly distinct.

3.1.1. Type species: *Umimayanthus chanpuru* sp. n. (Fig. 3 and Appendix A. Suppl. Fig. 3)

LSID: urn:lsid:zoobank.org:act:A7C6F356-9128-41EA-B108-271EC70B82FF

Synonymy. *Parazoanthus* sp. New Caledonia Deep1, *Parazoanthus* sp. New Caledonia Deep2 Sinniger et al. (2010); *Parazoanthus* sp. Reimer et al. (2014c).

Etymology. ‘Chanpuru’ from the Okinawan dialect of Japanese word “chanpuru” (チャンプル) meaning ‘mixed’ in reference to the variable morphological characters of the specimens included within this species.

Material examined. Type locality, for (#) refer to locations in Fig. 1:

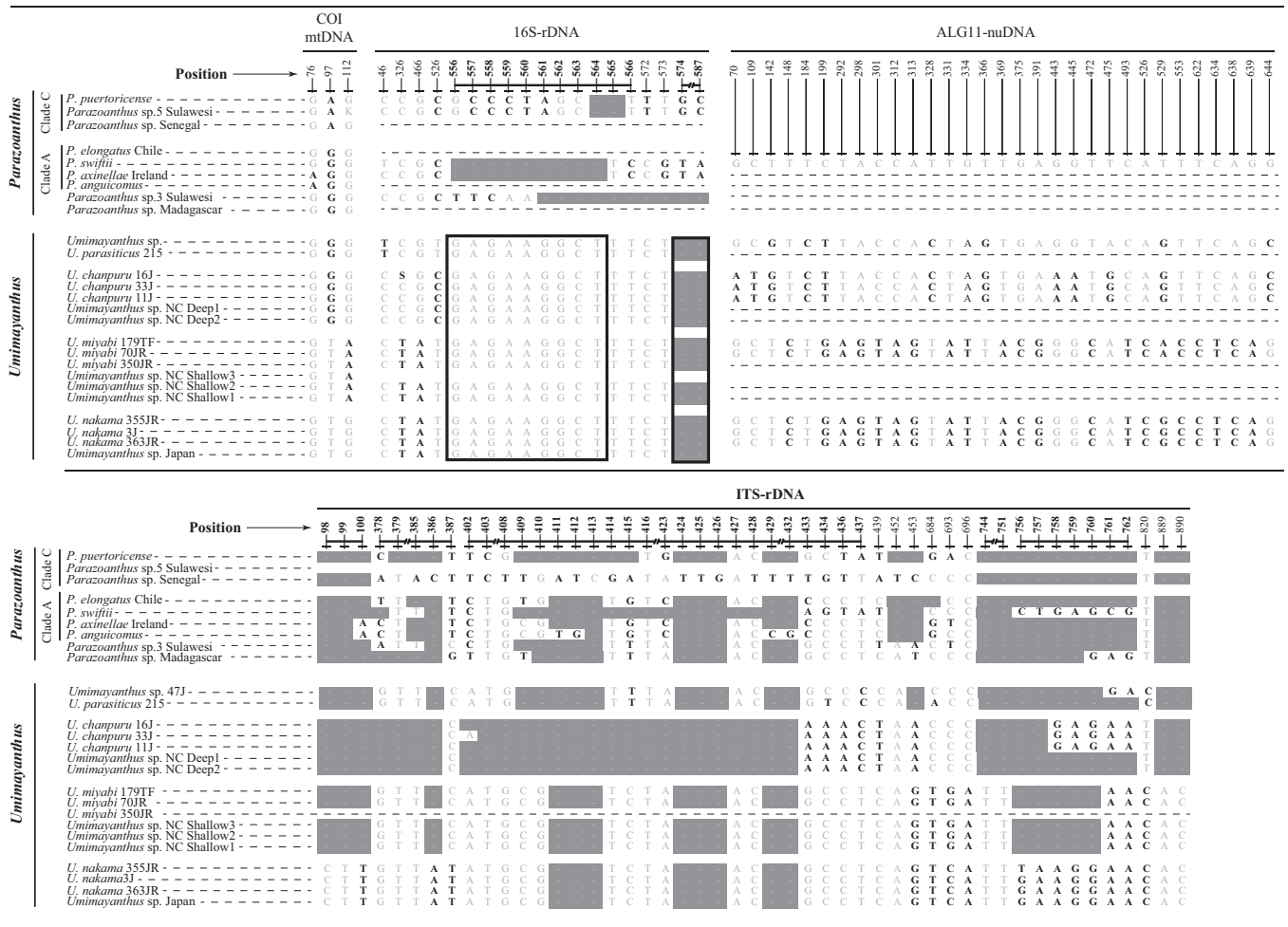


Fig. 2. Molecular characterization of *Umimayanthus* gen. n. species. Summary of deletions, insertions and base substitutions that characterize the different *Umimayanthus* gen. n. species among the genetic markers COI-mtDNA, mt 16S-rDNA, ITS-rDNA and ALG11-nuDNA. The black boxes in the mt 16S-rDNA region indicate the 9 bp long insertion and 14 bp deletion characteristics of the new genus *Umimayanthus*. Bold alignment positions indicate consecutive regions of genetic variation within the genetic markers.

Japan, Okinawa: Sesoko Island, Motobu, Sesoko Beach (1), 26°38'51"N/127°51'17"E; Motobu, Cape Bise (2), 26°42'44"N/127°52'43"E; Uruma, Ikei Island (3), 26°23'9"N/128°0'7"E; Kadena, Mizugama (4), 26°21'35"N/127°44'20"E; Kadena, Kadena Marina (5), 26°20'55"N/127°44'32"E; Kunigami, Yona (6), 26°46'4"N/128°11'49"E; Kunigami, Zatsun (7), 26°49'44"N/128°14'36"E; Maeda, Cape Maeda (8), 26°26'37"N/127°46'20"E; Onna, Manza (10), 26°30'11"N/127°50'35"E; Oura Bay, Nago, Kita-Nakase (11), 26°32'10"N/128°4'9"E; Oura Bay, Nago, Aosango (12), 26°32'14"N/128°4'34"E; Zamami Island, Yuhina (13), 26°14'30"N/127°18'23"E. Japan, Kagoshima, Yakushima, Sangohama, Kurio (14), 30°15'46"N/130°24'45"E. Japan, Yamaguchi, Oshima, Suo-Oshima (19), 33°56'3"N/132°10'49"E. Australia, Queensland, near Lizard Island: Reef 14–141 (20), 14°42'31"S/145°31'52"E; North Direction Island (21), 14°45'03"S/145°30'43"E; MacGillivray Reef (23), 14°39'29"S/145°29'30"E; Lagoon (24), 14°40'45"S/145°26'51"E; North Reef (25), 14°38'40"S/145°27'16"E.

Holotype: NSMT-Co1565 (MISE-JM-39J), 26°38'53"N 127°51'12"E, Sesoko Beach (1), Sesoko Island, Motobu, Okinawa, Japan, depth = 23 m, collected by J. Montenegro, date: April 26, 2011, fixed in 99% EtOH, deposited in National Museum of Nature and Science, Tokyo, Japan.

Paratypes (all from Okinawa, Japan).

Paratype 1: RUMF-ZG-04383 (MISE-JM-22J), 26°32'10"N, 128°4'9"E, Kita-Nakase (11), Oura Bay, Nago, Okinawa, Japan,

depth = 18 m, collected by J. Montenegro, date: March 3, 2011, fixed in 99% EtOH, deposited in Ryukyu University Museum, Fujikan.

Paratype 2: RMNH 41875 (MISE-JM-27J), 26°32'14"N, 128°4'34"E, Aosango (12), Oura Bay, Nago, Okinawa, Japan, depth = 16 m, collected by J. Montenegro, date: March 3, 2011, fixed in 99% EtOH, deposited in Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands.

Other material examined: All other specimens are deposited in the Molecular Invertebrate Systematics and Ecology (MISE) Laboratory collection at the University of the Ryukyus, Nishihara, Okinawa, Japan. The specimens' information is organized by localities.

Japan, Okinawa, Sesoko Island, Motobu, Sesoko Beach (1), 26°38'51"N/127°51'17"E: MISE-JM-40J, depth = 23 m, date April 26-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-38J, depth = 23 m, date April 26-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Motobu, Cape Bise (2), 26°42'44"N/127°52'43"E: MISE-JM-44J, depth = 25 m, date June 10-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-43J, depth = 25 m, date June 10-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Uruma, Ikei Island (3), 26°23'9"N/128°0'7"E: MISE-JM-42J, depth = 14 m, date April 29-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-41J, depth = 14 m, date April 29-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Kadena, Mizugama (4), 26°21'35"N/

127°44'20"E: MISE-JM-4J, depth = 8.5 m, date November 19-2010, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-5J, depth = 6.2 m, date December 11-2010, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-6J, depth = 6.2 m, date December 11-2010, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-30J, depth = 5 m, date March 14-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-31J, depth = 5 m, date March 14-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-32J, depth = 5 m, date March 14-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Kadena, Kadena Marina (5), 26°20'55"N/127°44'32"E: MISE-JM-56J, depth = 5 m, date September 27-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-57J, depth = 5 m, date September 27-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-58J, depth = 2 m, date September 27-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-59J, depth = 1.5 m, date September 27-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Kunigami, Yona (6), 26°46'4"N/128°11'49"E: MISE-JM-1J, depth = 10 m, date October 23-2010, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Kunigami, Zatsun (7), 26°49'44"N/128°14'36"E: MISE-JM-2J, depth = 10 m, date October 24-2010, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Maeda, Cape Maeda (8), 26°26'37"N, 127°46'20"E: MISE-JM-60J, depth = 19 m, date December 21-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-61J, depth = 21 m, date December 21-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Onna, Manza (10), 26°30'11"N/127°50'35"E: MISE-JM-15J, depth = 20 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-33J, depth = 19 m, date March 18-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-11J, depth = 18 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-8J, depth = 18 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-9J, depth = 18 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-10J, depth = 18 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-12J, depth = 17 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-13J, depth = 20 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-14J, depth = 18 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-34J, depth = 23 m, date March 18-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-35J, depth = 23 m, date March 18-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Oura Bay, Nago, Kita-Nakase (11), 26°32'10"N/128°4'9"E: MISE-JM-17J, depth = 18 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-21J, depth = 18 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-18J, depth = 18 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-16J, depth = 18 m, date February 8-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-19J, depth = 18 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-20J, depth = 18 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Oura Bay, Nago, Aosango (12), 26°32'14"N/128°4'34"E: MISE-JM-26J, depth = 16 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-24J, depth = 16 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-28J, depth = 16 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-23J, depth = 16 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-25J, depth = 16 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH; MISE-JM-29J, depth = 16 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Okinawa, Zamami Island, Yuhina (13), 26°14'30"N/127°18'23"E: MISE-JM-63J, depth = 13.3 m, date April 15-

2012, collected by J. Montenegro, fixed in 99% EtOH. Japan, Kagoshima, Yakushima, Sangohama, Kurio (14), 30°15'46"N/130°24'45"E: MISE-JDR/FS-529JR, depth = 12.8 m, date August 19-2008, collected by J.D. Reimer and F. Sinniger, fixed in 99% EtOH. Japan, Yamaguchi: Oshima, Suo-Oshima (19), 33°56'3"N/132°10'49"E: MISE-FI-61TF, depth = 15 m, date September 19-2011, collected by F. Iwase, fixed in 99% EtOH. Australia, Queensland, near Lizard Island, Reef 14-141 (20), 14°42'31"S/145°31'52"E: MISE-JDR-53JR, depth = 8 m, date August 26-2010, collected by J.D. Reimer, fixed in 99% EtOH. Australia, Queensland, near Lizard Island, North Direction Island (21), 14°45'03"S/145°30'43"E: MISE-JDR-62JR, depth = 12 m, date August 26-2010, collected by J.D. Reimer, fixed in 99% EtOH; MISE-JDR-54JR, depth = 8 m, date August 26-2010, collected by J.D. Reimer, fixed in 99% EtOH. Australia, Queensland, near Lizard Island, MacGillivray Reef (23), 14°39'29"S/145°29'30"E: MISE-JDR-122JR, depth = 6 m, date August 29-2010, collected by J.D. Reimer, fixed in 99% EtOH; MISE-JDR-123JR, depth = 5 m, date August 29-2010, collected by J.D. Reimer, fixed in 99% EtOH. Australia, Queensland, near Lizard Island, Lagoon (24), 14°40'45"S/145°26'51"E: MISE-JDR-121JR, depth = 6 m, date August 29-2010, collected by J.D. Reimer, fixed in 99% EtOH. Australia, Queensland, near Lizard Island, North Reef (25), 14°38'40"S/145°27'16"E: MISE-JDR-153JR, depth = 10 m, date August 31-2010, collected by J.D. Reimer, fixed in 99% EtOH.

Sequences. See [Appendix A. Suppl. Data 1](#).

Description. Size: The average polyp diameter is 0.85 ± 0.37 mm ($n = 113$). The colony size varies according to host sponge size.

Morphology: *Umimayanthus chanpuru* sp. n. has polyps that are yellow, orange or white. Polyps have 14–20 tentacles, longer than or equal to polyps' expanded oral disc diameter. Colonies consist exclusively of polyps homogeneously distributed over the sponge surface. Polyps can be solitary or connected by a stolon either over or under the sponge surface. Distance between polyps in colonies is 1.49 ± 1.10 mm ($n = 82$) on average (min. 0.04 mm and max. 5.37 mm; $n = 210$).

Cnidae: Spirocysts = avg. freq. 246 ($n = 24$); length range 11–22 μ m ($n = 95$); avg. length 16.2 ± 3 μ m ($n = 45$); b-mastigophores = avg. freq. 18 ($n = 24$); length range 11–21 μ m ($n = 91$); avg. length 16 ± 2.7 μ m ($n = 42$); p-mastigophores = avg. freq. 3 ($n = 24$); length range 12–20 μ m ($n = 46$); avg. length 16.7 ± 1.7 μ m ($n = 33$); basitrichs = avg. freq. 12 ($n = 24$); range 7–19 μ m ($n = 81$); avg. length 14.5 ± 2.9 μ m ($n = 38$); holotrichs-1 = avg. freq. 63 ($n = 24$); length range 17–32 μ m ($n = 68$); avg. length 24.1 ± 3.6 μ m ($n = 39$); holotrichs-2 = avg. freq. 7 ($n = 24$); length range 10–23 μ m ($n = 51$); avg. length 17.1 ± 3.2 μ m ($n = 30$).

Diagnosis. Polyp diameter 0.16–2.15 mm ($n = 442$). Sand content low to medium (0–2 on a scale of 0–3). Holotrichs-1 maximum size 32 μ m, largest size observed among all species of *Umimayanthus* gen. n. spp.

Habitat and distribution. *U. chanpuru* sp. n. is exclusively found in association with sponges of class Demospongia. Specimens were collected at 1.5–25 m depth. The coloration of host sponges varies between brown, green, grey, orange, pink, red, yellow, or white. The morphology of associated sponges is also variable and may be encrusting, cushion-shape, or massive. The encrusting and cushion-shape sponges were commonly found within cracks or holes in rocks or coral reefs, however massive sponges with *U. chanpuru* sp. n. were present in areas exposed to light.

U. chanpuru sp. n. specimens were found in southern Japan, the Great Barrier Reef (GBR) in Queensland, Australia, in the northern Red Sea (specimens reported in [Reimer et al., 2014c](#) as *Parazoanthidae*), and in New Caledonia (specimen GenBank sequence EU591578 in [Sinniger and Häussermann, 2009](#)), and likely the species is also distributed in the regions between these areas.

3.1.2. Species: *Uimayanthus miyabi* sp. n. (Fig. 4 and Appendix A. Suppl. Fig. 4)

LSID: urn:lsid:zoobank.org:act:6480A20E-1EEF-41A4-B582-4466770DF17F

Synonymy. *Parazoanthus* sp. New Caledonia Shallow1, *Parazoanthus* sp. New Caledonia Shallow2, *Parazoanthus* sp. New Caledonia Shallow3 in Sinniger et al. (2010).

Etymology. ‘Miyabi’ from the Japanese word “雅” meaning “elegance, refinement or courtliness” in reference to the delicate morphological appearance of the specimens included within this species.

Material examined. Type locality, for (#) refer to locations in Fig. 1:

Japan: Kagoshima, Yakushima, Hirauchi West (15), 30°13′55″N/130°30′7″E; Kochi, Otsuki, Nishidomari South (17), 32°46′27″N/132°43′53″E; Okinoshima, Murobae (27), 32°43′57″N/132°32′30″E; Okinawa: Taketomi, Iriomote Island (28), 24°25′28″N/123°48′30″E. Australia, Queensland, near Lizard Island: Outside Bird Islet (22), 14°41′01″S/145°27′01″E; Mermaid Cove (26), 14°38′51″S, 145°27′09″E.

Holotype. NSMT-Co1566 (MISE-JR-70JR), 14°41′01″S, 145°27′01″E, Outside Bird Islet (22), near Lizard Island, Queensland, Australia, depth = 1 m, collected by J.D. Reimer, date: August 26, 2010, fixed in 99% EtOH, deposited in National Museum of Nature and Science, Tokyo, Japan.

Paratypes

Paratype 1: RUMF-ZG-04384 (MISE-JR-354JR), 32°46′27″N, 132°43′53″E, Nishidomari South (17), Otsuki, Kochi, Japan, depth < 1.5 m, collected by J.D. Reimer, date: September 20, 2006, fixed in 99% EtOH, deposited in Ryukyu University Museum, Fujikan.

Paratype 2: RMNH 41876 (MISE-TF-179TF), 14°38′51″S, 145°27′09″E, Mermaid Cove (26), near Lizard Island, Queensland, Australia, depth = 12 m, collected by T. Fujii, date: September 2, 2010, fixed in 99% EtOH, deposited in Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands.

Other material: Other specimens are deposited in the Molecular Invertebrate Systematics and Ecology (MISE) Laboratory collection at the University of the Ryukyus, Nishihara, Okinawa, Japan. The specimens’ information is organized by localities.

Japan, Kagoshima, Yakushima, Hirauchi West (15), 30°13′55″N/130°30′7″E, MISE-JDR/FS-530JR, depth = 9 m, date August 19–2008, collected by J.D. Reimer and F. Sinniger, fixed in 99% EtOH. Japan, Kochi: Otsuki, Nishidomari South (17), 32°46′27″N/132°43′53″E: MISE-JDR-350JR, depth = <1 m, date September 20–2006, collected by J.D. Reimer, fixed in 99% EtOH; Okinoshima, Murobae (27), 32°43′57″N/132°32′30″E: MISE-JDR-324JR, depth = ~5 m, date March 22–2007, collected by J.D. Reimer, fixed in 99% EtOH. Japan, Okinawa, Taketomi, Iriomote Island (28), 24°25′28″N/123°48′30″E: MISE-JDR-16JR, depth = 6.6 m, date September 1–2012, collected by J.D. Reimer, fixed in 99% EtOH. Australia, Queensland, near Lizard Island, Outside Bird Islet (22), 14°41′01″S/145°27′01″E, MISE-JDR-70JR, depth = 1 m, date August 26–2010, collected by J.D. Reimer, fixed in 99% EtOH.

Sequences. See Appendix A. Suppl. Data 1.

Description. Size: The average polyp diameter is 1.07 ± 0.51 mm ($n = 10$). The colony size varies according to host sponge size.

Morphology: *Uimayanthus miyabi* sp. n. has pale yellow or white polyps. Polyps have 18–22 tentacles, longer than or equal to the length of expanded oral disc diameter. Colonies exclusively have polyps homogeneously distributed over the sponge surface. Distance between neighboring polyps: 1.95 ± 1.73 mm ($n = 10$) on average (min. 0.14 and max. 4.04 mm; $n = 90$).

Cnidae: Spirocysts = avg. freq. 411 ($n = 5$), range 8–18 μ m ($n = 23$), avg. length 14.4 ± 3.2 μ m ($n = 10$); b-mastigophores = avg. freq. 2 ($n = 5$); range 13–17 μ m ($n = 5$), avg. length 15.3 ± 2.1 μ m ($n = 3$); p-mastigophores = avg. freq. 1 ($n = 5$); range 14–14 μ m ($n = 2$), avg. length NA ($n = 2$); basitrichs = avg. freq. 11 ($n = 5$); range 13–17 μ m ($n = 6$), avg. length 15.2 ± 1.5 μ m ($n = 5$); holotrichs-1 = avg. freq. 55 ($n = 5$); range 18–24 μ m ($n = 20$), avg. length 21 ± 2.1 μ m ($n = 9$); holotrichs-2 = avg. freq. 24 ($n = 5$); range 9–19 μ m ($n = 13$), avg. length 14.2 ± 3.2 μ m ($n = 9$).

Differential diagnosis. Polyp diameter 0.29–1.70 mm ($n = 113$). Different from *U. chanpuru* sp. n., *Uimayanthus miyabi* sp. n. exclusively associates with massive sponges. Colonies are always formed by solitary polyps scattered over the host sponge surface, and the sand content in tissues is always medium or high (2–3 in scale of 0–3). Additionally, in cnidae analyses the maximum size of holotrichs-1 was 24 μ m, smaller and easily distinguishable (Fig. 5) from *U. chanpuru* sp. n.

Habitat and distribution. *U. miyabi* sp. n. is exclusively found in association with sponges, and specimens were collected from depths of 1–12 m. The host sponges were usually orange or occasionally purple, and all were massive in morphology. Different from *U. chanpuru* sp. n., *Uimayanthus miyabi* sp. n. was never seen inhabiting sponges found in cracks or holes, but was instead always found on sponges in exposed conditions. *U. miyabi* sp. n. is present in southern Japan, New Caledonia and in Queensland (GBR), Australia, and it is likely that the species is distributed in Indo-Pacific regions between these two areas.

Remarks: There is a *Parazoanthus* sp. reported in Sinniger, 2007 that may refer to *U. miyabi*, but further molecular and morphological analyses are required to properly confirm its identity.

3.1.3. Species: *Uimayanthus nakama* sp. n. (Fig. 6 and Appendix A. Suppl. Fig. 5)

LSID: urn:lsid:zoobank.org:act:95F97481-13F3-4E7C-8F70-A444A158349C

Synonymy. *Parazoanthus* sp. Japan, Sinniger et al. (2010).

Etymology. ‘Nakama’ from the Japanese word ‘仲間’ meaning ‘friends’ or ‘associates’ in reference to the commonly seen arrangement of polyps in groups of two or three.

Material examined. Type locality, for (#) refer to locations in Fig. 1:

Japan, Okinawa: Motobu, Sesoko Island (1), 26°38′51″N/127°51′17″E; Kadena, Mizugama (4), 26°21′35″N/127°44′20″E; Nago, Teniya (9), 26°33′56″N/128°8′25″E. Japan, Kochi, Otsuki: Nishidomari South (17), 32°46′27″N/132°43′53″E; Odo North (18), 32°46′53″N/132°40′09″E.

Holotype: NSMT-Co1567 (MISE-DU-60U), 26°38′53″N/127°51′12″E Sesoko Island (1), Motobu, Okinawa, Japan, depth = 25 m, collected by D. Uyeno, date: November 7, 2011, fixed in 99% EtOH, deposited in National Museum of Nature and Science, Tokyo, Japan.

Paratypes (all from Okinawa, Japan)

Paratype 1: RMNH 41877 (MISE-JR-365JR), 32°46′53″N, 132°40′09″E, Odo North (18), Otsuki, Kochi, Japan, depth = 3 m, collected by J.D. Reimer, date: September 13, 2006, fixed in 99% EtOH, deposited in Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands.

Paratype 2: RUMF-ZG-04385 (MISE-JR-355JR), 32°46′27″N, 132°43′53″E, Nishidomari South (17), Otsuki, Kochi, Japan, depth < 1.5 m, collected by J.D. Reimer, date: September 20, 2006, fixed in 99% EtOH, deposited in Ryukyu University Museum, Fujikan.

Other material: Other specimens are deposited in the Molecular Invertebrate Systematics and Ecology (MISE) Laboratory collection at the University of the Ryukyus, Nishihara, Okinawa, Japan. The specimens’ information is organized by localities.

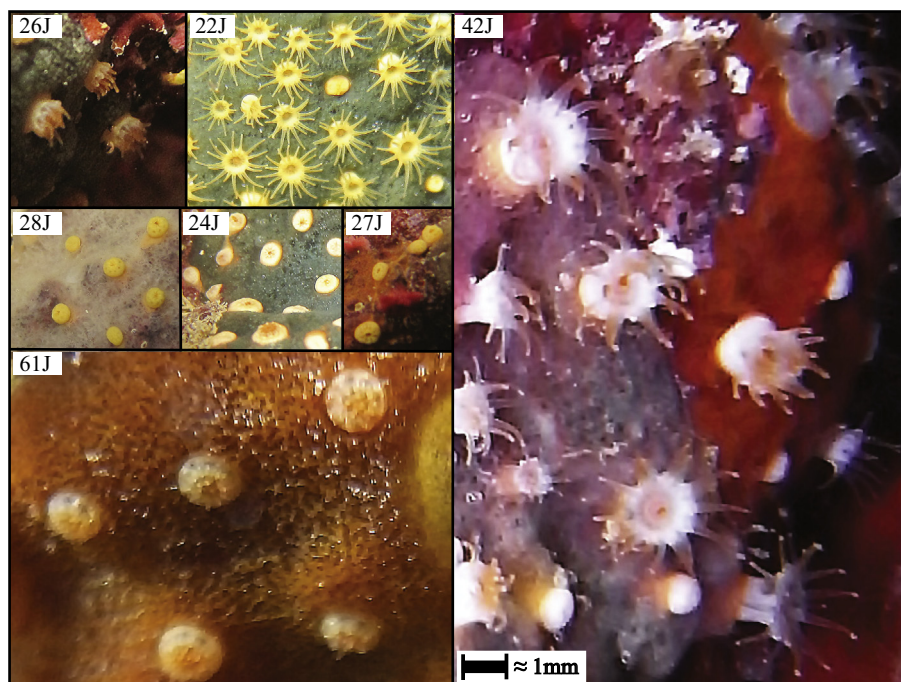


Fig. 3. In-vivo pictures of seven different colonies of *Uimayanthus chanpuru* sp. n.

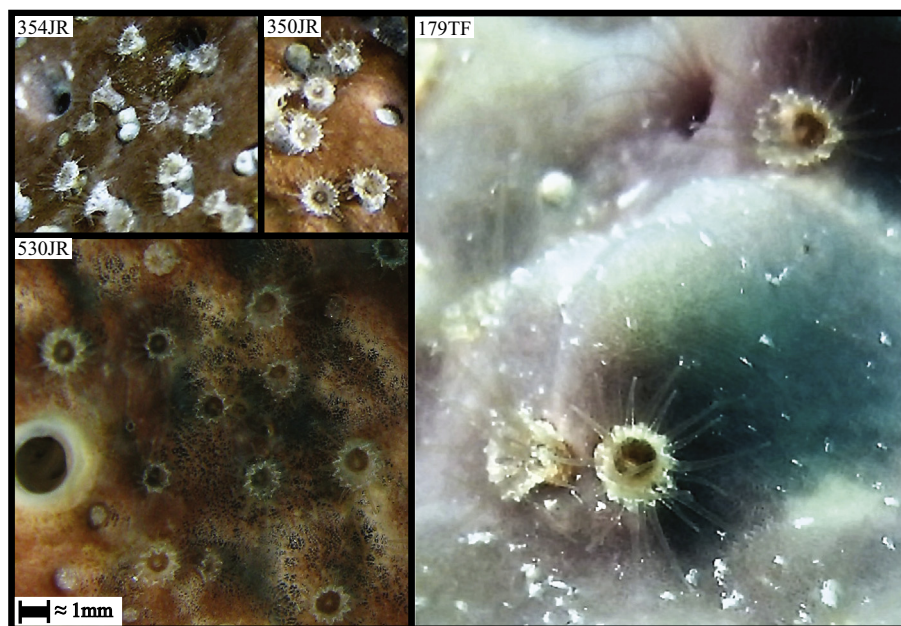


Fig. 4. In-vivo pictures of four different colonies of *Uimayanthus miyabi* sp. n.

Japan, Okinawa: Motobu, Sesoko Island (1), 26°38'51"N/127°51'17"E, MISE-DU-60U, depth = 25 m, date November 7-2011, collected by D. Uyeno, fixed in 99% EtOH. Kadena, Mizugama (4), 26°21'35"N/127°44'20"E, MISE-JM-3J, depth = 8.5 m, date November 19-2010, collected by J. Montenegro, fixed in 99% EtOH. Nago, Teniya (9), 26°33'56"N/128°8'25"E, MISE-JM-37J, depth = 12 m, date March 3-2011, collected by J. Montenegro, fixed in 99% EtOH. Japan, Kochi, Otsuki, Odo North (18), 32°46'53"N/132°40'09"E, MISE-JDR-363JR, depth = 3 m, date September 13-2006, collected by J.D. Reimer, fixed in 99% EtOH.

Sequences. See [Appendix A. Suppl. Data 1](#).

Description. Size: The average polyp diameter is 1.38 ± 0.64 mm ($n = 12$). The colony size varies according to the host sponge size.

Morphology: *Uimayanthus nakama* sp. n. has pale orange or white polyps. Polyps have approximately 20–22 tentacles. Colonies consist of both solitary polyps and polyps in groups of two or three polyps, scattered over the sponge surface. Distance between neighboring polyps within a colony is 1.48 ± 1.35 mm ($n = 12$) on average (min. = joined and max. 4.42 mm; $n = 59$).

Cnidae: Spirocysts = avg. freq. 302 ($n = 6$), range 13–21 μ m ($n = 23$), avg. length 16 ± 2.2 μ m ($n = 12$); b-mastigophores = avg.




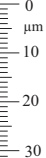
Cnidocyst	Umimayanthus															Scale (40x)
	U. chanpuru					U. miyab i					U. nakama					
Holotrichs-1																
	Freq.	Min.	Max.	Ave.	SDV	Freq.	Min.	Max.	Ave.	SDV	Freq.	Min.	Max.	Ave.	SDV	
	63	17	32	24.1	3.6	55	18	24	21	2.1	5	17	21	19.2	1.5	

Fig. 5. Results of cnidocyte analyses of the holotrich-1 category among different phylogenetic clades. Notice the large differences between clade *U. chanpuru* vs. *U. miyabi*/*U. nakama* in holotrich-1 sizes.

freq. 12 ($n = 6$), range 14–18 μm ($n = 20$), avg. length $15.2 \pm 1.3 \mu\text{m}$ ($n = 12$); p-mastigophores = avg. freq. 2 ($n = 6$), range 13–17 μm ($n = 8$), avg. length $15 \pm 1.4 \mu\text{m}$ ($n = 7$); basitrichs = avg. freq. 13 ($n = 6$), range 11–16 μm ($n = 16$), avg. length $13.8 \pm 1.5 \mu\text{m}$ ($n = 10$); holotrichs-1 = avg. freq. 5 ($n = 6$), range 17–21 μm ($n = 6$), avg. length $19.2 \pm 1.5 \mu\text{m}$ ($n = 5$); holotrichs-2 = avg. freq. 13 ($n = 6$), range 10–19 μm ($n = 13$), avg. length $16 \pm 3.1 \mu\text{m}$ ($n = 7$).

Differential diagnosis. Polyp diameter 0.62–2.3 mm ($n = 86$), notably bigger than those of *U. chanpuru* sp. n. and *U. miyabi* sp. n. Unlike *U. chanpuru* sp. n. and *U. miyabi* sp. n., *Umimayanthus nakama* sp. n. exclusively associates with sponges of encrusting or cushion morphologies; the colonies are formed by solitary polyps or by groups of two to three polyps scattered over the sponge surface; sand content in tissues is always high (3 on a scale of 0–3). Additionally, in cnidae analyses the maximum size of holotrichs-1 was 21 μm , easily distinguishable (Fig. 5) from the larger sizes seen in *U. chanpuru* sp. n.

Habitat and distribution. *U. nakama* sp. n. is exclusively found in association with sponges, and specimens were collected from depths of 1.5 to 25 m. The associated sponges were yellow, green, white or brown, and encrusting or cushion in morphology. *U. nakama* sp. n. was found on sponges inhabiting cracks on coral reefs or in exposed conditions. *U. nakama* sp. n. specimens were only found in southern Japan.

Remarks: *U. nakama* is similar to *P. lividum* Cutress, 1971 found off Williamstown, Victoria, Australia, but the two species are distinguishable as *P. lividum* has 29 tentacles, polyps moderately incrustated with sand, and a maximum holotrich size of 12 μm , while *U. nakama* has at maximum 22 tentacles, polyps saturated with sand, and a maximum holotrich size of 21 μm . As well, *P. lividum* was found colonizing an unidentified sponge of the genus *Spirasterella* (family Spirastrellidae); sponges of this family are characterized by presenting a exhalant vein-like canal system evident on the surface and converge on an oscula (Rützler, 2002), a characteristic not present in any of the host sponges colonized by *U. nakama*.

However, given general morphological similarities and location in the Indo-Pacific, in the future based on molecular analyses it is conceivable that *P. lividum* may be transferred to genus *Umimayanthus*.

4. Molecular characterization of *Umimayanthus* gen. n. species

For molecular characterization of *Umimayanthus* gen. n. and species within, only alignments not edited with GBlock were analyzed and described below. All positions refer to positions in alignments used in this study. As indicated in Section 3.1, species in *Umimayanthus* gen. n. have a highly conservative and unique insertion of 9 bp in length (from position 556 to 564) and one deletion of 14 bp in length (from position 574 to 587) in the mt 16S-rDNA alignment (Fig. 2).

U. miyabi and *U. nakama* can be differentiated from *U. chanpuru* in the COI-mtDNA alignment by a unique and consistent base substitution of “G” for “T” at position 97. As well, *U. miyabi* can be distinguished from *U. nakama* by a substitution of “G” for “A” at position 112 (Fig. 2).

U. miyabi and *U. nakama* were different from *U. chanpuru* and *U. parasticus* in the mt 16S-rDNA region by two consistent base substitutions; “C” for “T” at position 326 and “G” for “A” at position 466 (Fig. 2).

U. chanpuru, *U. miyabi* and *U. nakama* were clearly different in the ITS-rDNA alignment by several insertions, deletions, and base substitutions throughout the whole rDNA region (Fig. 2). For example, *U. nakama* is separated from the other species in *Umimayanthus* and *Parazoanthus* by a unique insertion of 3 bp long from 98 to 100. *U. miyabi* and *U. chanpuru* can be differentiated by a unique combination of deletions and insertions in the ITS-rDNA alignment as shown in Fig. 2 (alignments available from the corresponding author upon request).

Similar to 16S-rDNA alignments, in sequences of ALG11-nuDNA, *U. miyabi* and *U. nakama* can be distinguished from *U. chanpuru* by several single base substitutions as indicated in Fig. 2.

The sequences of *Parazoanthus* sp. Madagascar and *Parazoanthus* sp.3 Sulawesi from Sinniger et al. (2010) were always within or close to *Umimayanthus* in the phylogenetic analyses. These sequences were ‘singletons’ inside the *Umimayanthus* gen. n. clade (ITS-rDNA) or closely related (COI-mtDNA and 16S-rDNA) to *Umimayanthus* gen. n. Despite been phylogenetically close to *Umimayanthus* gen. n., the sequence of *Parazoanthus* sp.3 Sulawesi did not have the 9 bp long (position 556–564) insertion characteristic of *Umimayanthus* gen. n., but instead a 27 bp long deletion (from position 561 to 587) that overlapped with the characteristic 14 bp (position 574–587) deletion of *Umimayanthus* gen. n. (Fig. 2) in the 16S-rDNA region. Given the lack of conclusive molecular data and the absence of morphological information we have refrained from including *Parazoanthus* sp. Madagascar and *Parazoanthus* sp.3 Sulawesi from Sinniger et al. (2010) inside *Umimayanthus* gen. n. in this study.

5. Results

5.1. Specimen collection

The sponge-parazoanthid association was observed to be widely distributed and common in southern Japanese waters (Fig. 1). For most sampled specimens the associated sponges were encrusting or cushion-shaped, with the exception of specimens 34J (Okinawa, associated with massive sponge), 324JR, 350JR, 354JR, 530JR, and 179TF (Australia, associated with massive sponges). Most specimens were found inside cracks or holes in

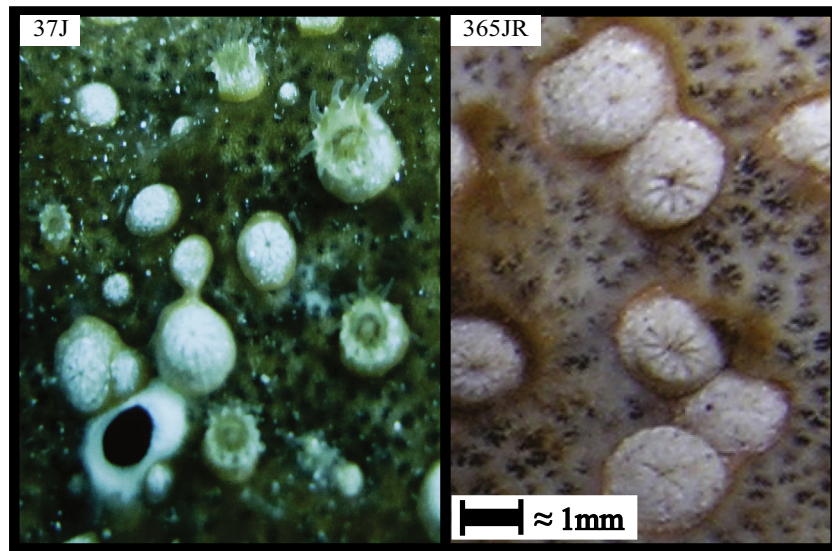


Fig. 6. In-vivo picture of a colony of *Uimimayanthus nakama* sp. n.

rocks or reefs, and when specimens were found in caves or limited light areas they were generally midway between cave entrances and the darkest areas.

5.2. Phylogenetic analyses

5.2.1. Phylogeny of ITS-rDNA region

The phylogenetic reconstruction using the strict alignment of the ITS-rDNA region is shown in Fig. 7. Sequences of *Antipathozoanthus macaronesicus* (GenBank: EU591556, EU591552) were used as outgroup. The sequences of *P. darwini* and *P. swiftii* (Duchassaing and Michelotti, 1860) branched together in a separated clade basal to all the other sequences with strong support (ML = 100%; NJ = 100%; BPP = 1). Sequences of *P. elongatus*, *P. anguicomus* (Norman, 1868) and *P. axinellae* were contained in a well-supported clade (ML = 85%; NJ = 91%; BPP = 1) basal to genus *Uimimayanthus* and a derivate clade (support: ML = 76%; NJ = 97%; BPP = 0.98) consisting of sequences of *P. puertoricense*, *P. catenularis* (Duchassaing and Michelotti, 1860), and the single sequence of an undescribed *Parazoanthus* sp. from Senegal (GenBank: EU591582).

All sequences belonging to genus *Uimimayanthus* were contained in well-supported clade (ML = 72%; NJ = 99%; BPP = 0.99). One large clade was formed by nine sequences (ML = 95%; NJ = 99%; BPP = 1) that were divided into two sister subclades with moderate support (ML = 51%; NJ = 98%; BPP = 0.61; and ML = 76%; NJ = 66%; BPP = 0.99, respectively), consisting of *U. nakama* and *U. miyabi* sequences, respectively. These subclades were basal to sequences from two undescribed *Parazoanthidae* specimens (*Parazoanthus* sp. Madagascar 3, GenBank: EU591576; *Parazoanthus* sp.3 Sulawesi, GenBank: EU591575), and to sequences of specimen 47J and another clade, consisting of *U. chanpuru* sequences. The sequences of samples 47J and *U. parasiticus* formed an impendent clade with strong support (ML = 88%; NJ = 96%; BPP = 1).

All sequences belonging to *U. chanpuru* were contained within a single clade with strong support (ML = 94%; NJ = 99%; BPP = 1). Inside *U. chanpuru* there were seven independent clades with moderate to strong support (ML = >53%; NJ = >55%; BPP = >0.68).

5.2.2. Phylogeny of COI-mtDNA region

The phylogenetic reconstruction using the strict alignment of the COI-mtDNA region is shown in Fig. 8. Sequences of

Antipathozoanthus macaronesicus (GenBank: EU591618, AB247357) were used as outgroup. *U. nakama* (ML = 62%; NJ = 62%; BPP = 0.89) was basal to a subclade formed by *U. miyabi* (ML = 64%; NJ = 63%; BPP = 0.88), and both of these were derived from a major clade containing sequences from genera *Parazoanthus* and *Uimimayanthus*.

The clade containing sequences of *Parazoanthus* and *Uimimayanthus* (largely consisting of *U. chanpuru* sequences) was moderately supported (ML = 61%; NJ = 72%; BPP = 0.99), and contained the majority of specimens' sequences (52 sequences). This large clade contained four singletons (specimens 61TF, 44J, 36J and 29J) and three subclades: sequences of *P. elongatus* (GenBank: EF672662, EF672661) formed a separate subclade with fairly strong support (ML = 86%; NJ = 86%; BPP = 1); sequences of *P. swiftii* split into a separate clade with moderate support (ML = 61%; NJ = 63%; BPP = 0.69); and finally sequences of *P. anguicomus* (GenBank: EF672660) along with six specimens' sequences (1J, 39J, 17J, 20J, 24J, FS706) and two sequences of *P. axinellae* formed a moderately supported subclade (ML = 64%; NJ = 63%; BPP = 0.92), where the sequences of *P. axinellae* (GenBank: EF672659, AB247355) branch in a separated clade with moderate support (ML = 64%; NJ = 64%; BPP = 0.96).

5.2.3. Phylogeny of mt 16S-rDNA region

The phylogenetic reconstruction of the mt 16S-rDNA alignment is shown in Fig. 9. Sequences of *Antipathozoanthus macaronesicus* (Ocana and Brito, 2003; GenBank: AY995932, AY995931) were used as outgroup. All sequences belonging to *Parazoanthus* and *Uimimayanthus* were together in a single big clade with complete support (ML = 100%; NJ = 100%; BPP = 1).

Sequences of *P. axinellae*, *P. darwini* and *P. swiftii* branched in a separate clade with moderate support (ML = 87%; NJ = 67%; BPP = 1). *P. axinellae* was basal to a subclade containing all sequences of *P. swiftii* and *P. darwini* (ML = 83%; NJ = 84%; BPP = 1). The sequence of *Parazoanthus* sp.3 Sulawesi (GenBank: AY995937; Sinniger et al., 2010) was weakly supported as basal to a strongly supported clade (ML = 97%; NJ = 92%; BPP = 1) containing a single sequence of *Parazoanthus* sp.5 Sulawesi (GenBank: AY995934; Sinniger et al., 2010) and a well-supported (ML = 93%; NJ = 92%; BPP = 1) subclade formed by sequences of *P. catenularis* and *P. puertoricense*.

The single sequence from specimen 47J was weakly supported as sibling to the sequences of *U. parasiticus*. These sequences were

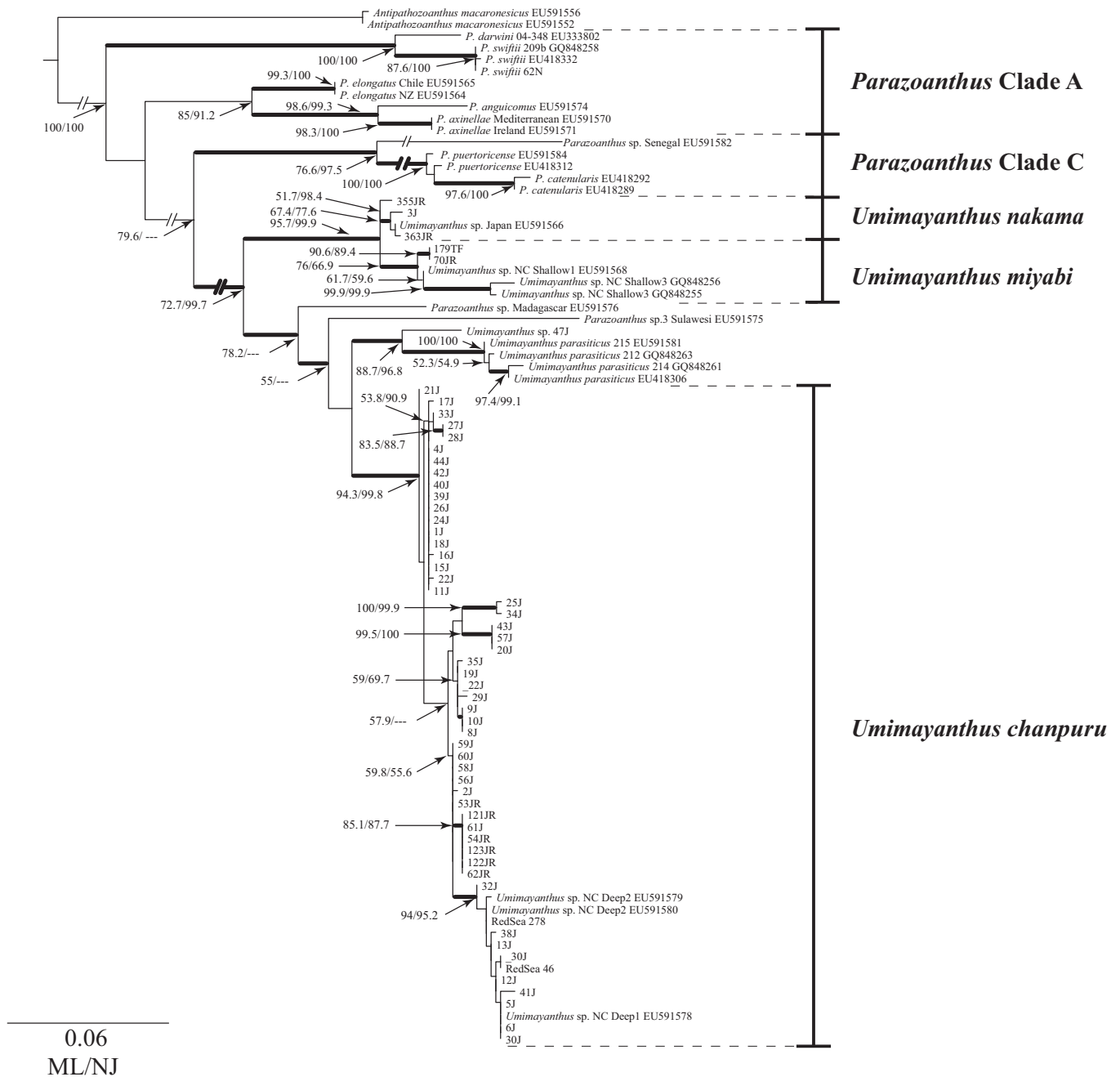


Fig. 7. Maximum-likelihood phylogenetic cladogram tree obtained using strict alignments of ITS-rDNA sequences edited by GBlock. Values at branches represent bootstrap NJ and ML values over 50% for the samples studied. Bayesian posterior probabilities over 0.95 are represented by thick branches. For Genbank accession numbers, names and abbreviations of the sequences used in phylogenetic trees see [Appendix A. Suppl. Data 1 and 2](#).

sister to a multispecies group formed by specimens belonging to *U. miyabi* and *U. nakama*. This multispecies group was formed by 15 sequences with moderate support (ML = 72%; NJ = 62%; BPP = 0.68). *U. chanpuru* was weakly supported as a monophyly and was formed by the majority of specimens' sequences (49 sequences).

5.2.4. Phylogeny of ALG11-nuDNA region

The phylogenetic reconstruction using the strict alignment of ALG11-nuDNA region is shown in [Fig. 10](#). The sequence of *P. swiftii* (62N) was used as outgroup. *U. miyabi* and *U. nakama* were collapsed into a single multispecies group formed by nine sequences with very high support (ML = 100%; NJ = 100%; BPP = 1) and basal to sequence of the specimen 47J and to *U. chanpuru*.

The sequence from specimen 47J was presented as sister to *U. chanpuru* with strong support (ML = 87%; NJ = 96%; BPP = 0.63). *U. chanpuru* was formed by the majority of specimens' sequences with moderate support (ML = 85%; NJ = 81%, B = 0.73). *U. chanpuru* included three well-supported subclades.

5.3. Morphological analyses

The results of one-way ANOVA and Kruskal–Wallis tests did not show any significant differences existed across *Uimayanthus* species regarding sizes of spirocysts, b-mastigophores, basitrichs, and holotrichs-2. However, there were significant differences in minimum and maximum sizes of holotrichs-1 (I-ANOVA minimum size DF = 30, $f = 4.365$, $p = 0.022$; Kruskal–Wallis maximum size DF = 2,

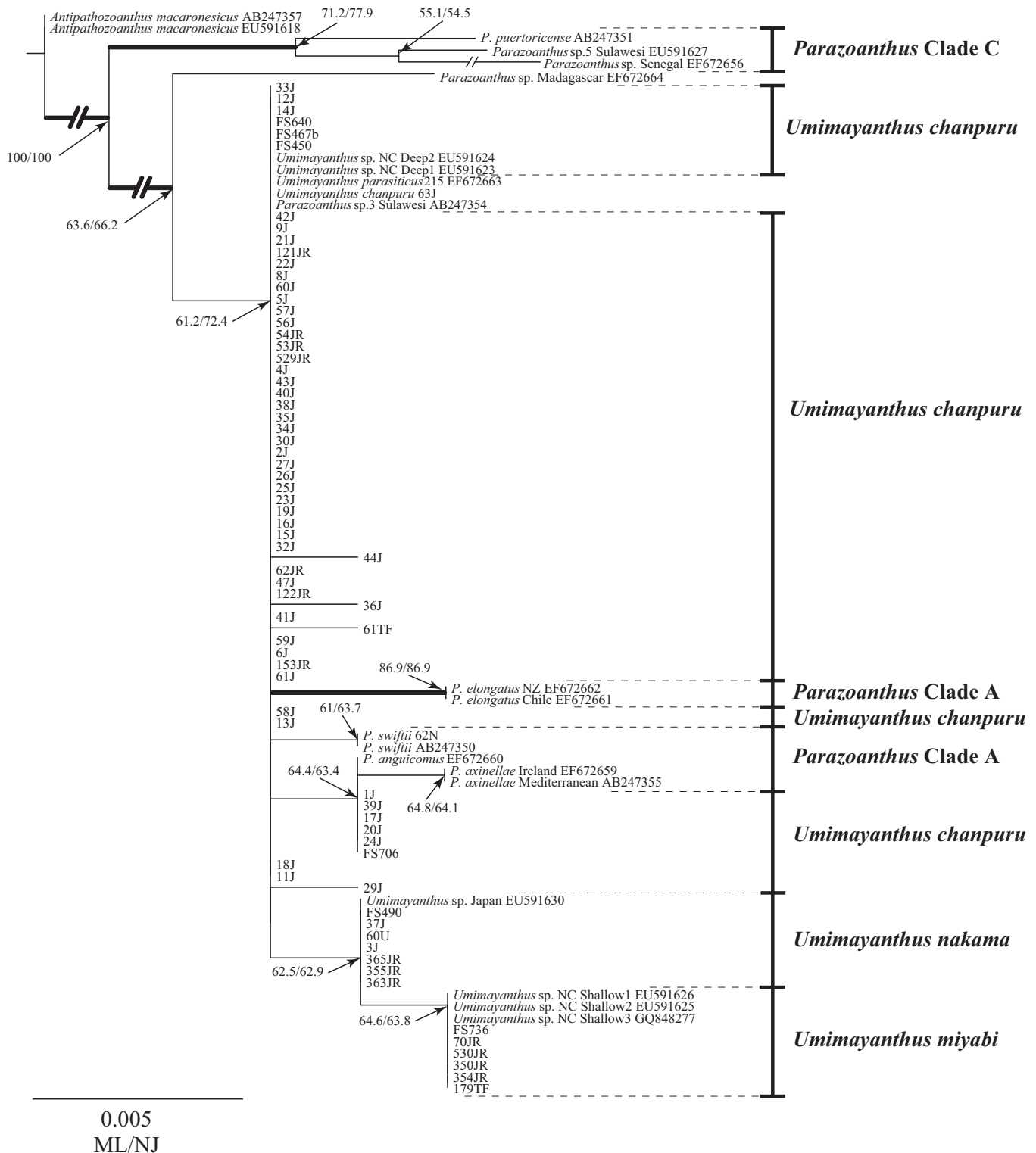


Fig. 8. Maximum-likelihood phylogenetic cladogram tree obtained using strict alignments of COI-mtDNA sequences edited by GBlock. Values at branches represent bootstrap NJ and ML values over 50% for the samples studied. Bayesian posterior probabilities over 0.95 are represented by thick branches. For Genbank accession numbers, names and abbreviations of the sequences used in phylogenetic trees see [Appendix A. Suppl. Data 1 and 2](#).

K-observed = 10.358, K-critical = 5.991, $p = 0.006$; Fig. 5), and p-mastigophores (I-ANOVA minimum size $DF = 23$, $f = 5.599$, $p = 0.027$; maximum size $DF = 23$, $f = 8.121$, $p = 0.009$). For the independent two-sample t -test results from the post hoc analyses of the cnidae data, see Table 1.

There were also significant differences among *Umimayanthus* species' size range of polyps (I-ANOVA minimum size DF = 67, $f = 3.376$, $p = 0.040$; maximum size DF = 67, $f = 14.289$, $p < 0.001$), distances between polyps (I-ANOVA minimum distance DF = 62, $f = 4.69$, $p = 0.013$; maximum distance DF = 62, $f = 5.507$,

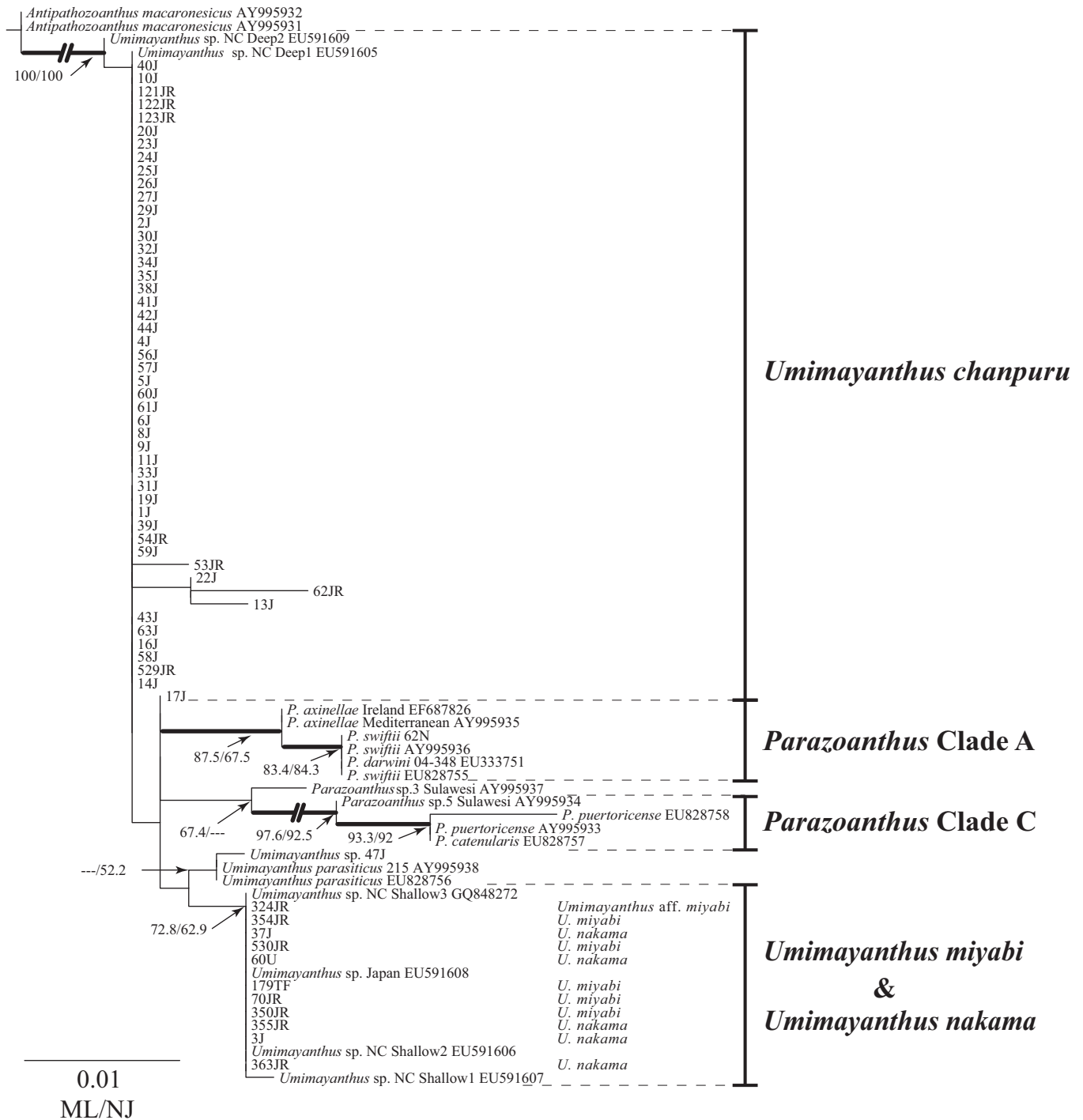


Fig. 9. Maximum-likelihood phylogenetic cladogram tree obtained using strict alignments of 16S-rDNA sequences edited by GBlock. Values at branches represent bootstrap NJ and ML values over 50% for the samples studied. Bayesian posterior probabilities over 0.95 are represented by thick branches. For Genbank accession numbers, names and abbreviations of the sequences used in phylogenetic trees see [Appendix A. Suppl. Data 1 and 2](#).

$p = 0.006$), and in sand content of tissue (Kruskal–Wallis $DF = 2$, K -observed = 8.588, K -critical = 5.991, $p = 0.014$). For the independent two-sample t -test results see [Table 2](#).

6. Discussion

Although the genus *Umimayanthus* has some morphological and ecological similarities with genus *Parazoanthus* sensu stricto, there are also key differences. *Umimayanthus* was described in [Sinniger](#)

[et al. \(2010\)](#) (as *Parazoanthus* clade B) as “...small polyps linked together through stolons that may sometimes be absent altogether. The polyps are usually scattered on the surface of the sponge...”; characteristics shared by all specimens examined in this study. *Umimayanthus* spp. have polyp diameters ranging from 0.16–2.3 mm ($n = 641$), in contrast to *Parazoanthus* sensu stricto spp. (clade A) with a bigger polyp diameters ranging from 2 to 10 mm, and *Parazoanthus* spp. (clade C) with polyp diameters ranging from 1 to 1.5 mm. *Umimayanthus* can be easily distinguished from *Parazoanthus* sensu stricto spp. (Clade A), but may be

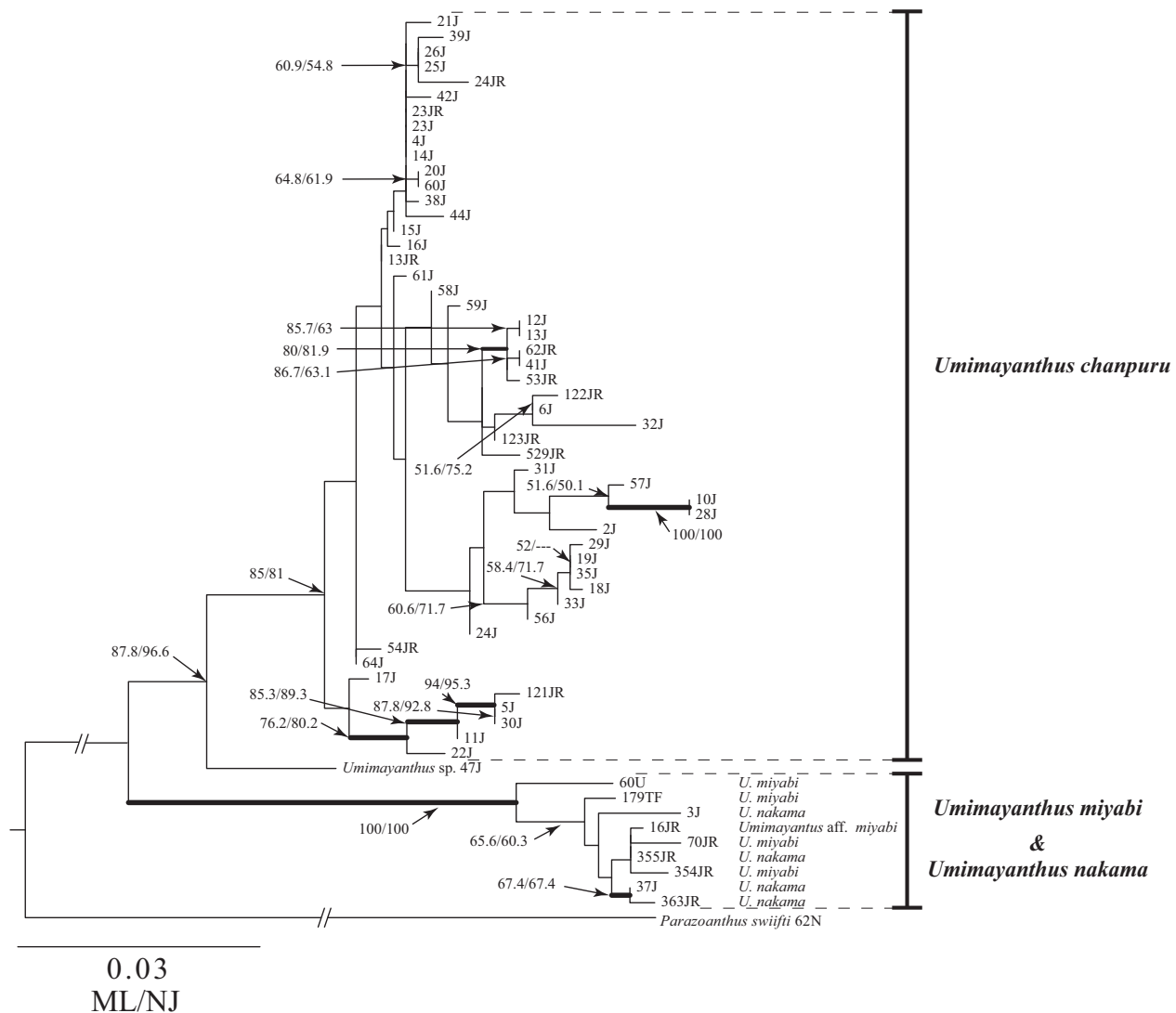


Fig. 10. Maximum-likelihood phylogenetic cladogram tree obtained using strict alignments of ALG11-nuDNA sequences edited by GBlock. Values at branches represent bootstrap NJ and ML values over 50% for the samples studied. Bayesian posterior probabilities over 0.95 are represented by thick branches. For Genbank accession numbers, names and abbreviations of the sequences used in phylogenetic trees see [Appendix A. Suppl. Data 1](#).

confused with *Parazoanthus* spp. (Clade C) given polyp sizes and geographical distribution. Currently, all formally described species of *Parazoanthus* spp. (clade C) are native to the North Atlantic Ocean, but there is an undescribed *Parazoanthus* (clade C) specimen from Sulawesi (*Parazoanthus* sp.5 Sulawesi; Sinniger et al., 2010; Table 3).

With the exception of *U. parasiticus*, genus *Umimayanthus* appears to be mostly limited to the Indo-Pacific, differently from *Parazoanthus* (Table 3) that has a much wider distribution. Currently, seven species of sponge-associated *Parazoanthus* spp. are described from the Atlantic Ocean, six species in the Pacific and Indian Oceans, and two species in the Arctic and Antarctic Oceans. On the other hand, three of four described *Umimayanthus* spp. as well as all other undescribed specimens are from the Indo-Pacific, and all species have subtropical–tropical distribution. Future investigation and formal descriptions of species from other regions may help to corroborate or refute this potential biogeographical pattern.

Additionally, from this and other recent work in southern Japan and the Pacific, it is apparent that species in the new genus *Umimayanthus* have some differences in habitat compared to *Parazoanthus* sensu stricto. In the Caribbean Sea, *Parazoanthus*

sensu stricto is often found in areas with relatively high light exposure associated to sponges with different morphologies (Swain and Wulff, 2007; Montenegro and Acosta, 2010). However, in this study the specimens from the new genus *Umimayanthus* were found primarily in areas with low light exposure, such as in cracks, hollows and caves, and associated mainly with encrusting or cushion-shape sponges. Additionally, the three new species here may have differing distribution ranges within the Pacific; *U. chanpuru* was found from Yamaguchi, Kagoshima, and Okinawa prefectures in Japan, from Queensland (GBR) in Australia, the Red Sea in Saudi Arabia, and from New Caledonia in France; *U. miyabi* also similarly appears to have a wide distribution with specimens from Kochi and Kagoshima prefectures in Japan, from New Caledonia, and from Queensland (GBR) in Australia; while *U. nakama* was only found in Japan, from Kochi to Okinawa. Future work on the marine regions between Japan and Australia should help to delineate the ranges of *Umimayanthus* species.

The results of our phylogenetic analyses in the COI-mtDNA region clearly differentiated the two species *U. miyabi* and *U. nakama* from all other sequences, but failed to discriminate sequences of *U. chanpuru* from the sequences of specimen 47J, *Parazoanthus* sp.3 Sulawesi (GenBank: AB247354) and from species

Table 1Statistical analyses of cnidocyst data comparing three new species of *Umimayanthus* gen. n.

			ANOVA/Kruskal–Wallis	Independent two-sample <i>T</i> -test/Cochran–Cox <i>T</i> -test <i>Umimayanthus</i>		
				<i>U. chanpuru</i> – <i>U. miyabi</i>	<i>U. chanpuru</i> – <i>U. nakama</i>	<i>U. miyabi</i> – <i>U. nakama</i>
<i>Cnidocyst categories</i>						
Spirocyst	Size	Frequency	<u>0.476</u>	<u>0.288</u>	<u>0.748</u>	<u>0.615</u>
		Minimum	0.152	0.125	0.425	0.099
		Maximum	0.052	0.024*	0.174	0.427
b-Mastigophores	Size	Frequency	0.075	0.036*	0.348	0.095
		Minimum	<u>0.687</u>	< <i>n</i>	<u>0.824</u>	< <i>n</i>
		Maximum	0.068	< <i>n</i>	0.068	< <i>n</i>
Basitrichs	Size	Frequency	0.935	0.873	0.781	0.699
		Minimum	0.21	0.108	0.9	0.044*
		Maximum	0.148	0.539	0.065	0.215
Holotrichs-1	Size	Frequency	<u>0.064</u>	<u>0.82</u>	<u>0.015</u> *	<u>0.108</u>
		Minimum	<u>0.022</u> *	0.034*	0.050*	0.645
		Maximum	<u>0.006</u> *	<u>0.002</u> *	<u><0.001</u> *	<u>0.027</u> *
Holotrichs-2	Size	Frequency	0.192	0.066	0.482	0.427
		Minimum	0.136	0.055	0.325	0.553
		Maximum	0.139	0.057	0.28	0.642
p-Mastigophores	Size	Frequency	0.193	0.128	0.316	0.17
		Minimum	0.027*	< <i>n</i>	0.027*	< <i>n</i>
		Maximum	0.009*	< <i>n</i>	0.009*	< <i>n</i>

* The *p*-values with a significant statistical difference. Underlined *p*-values correspond to Kruskal–Wallis and Cochran–Cox correction for the *T*-test. <*n* = sample size smaller or equal than two.

Table 2Statistical analyses of external morphology comparing three new species of *Umimayanthus* gen. n.

			ANOVA	Independent two-sample <i>T</i> -test <i>Umimayanthus</i>		
				<i>U. chanpuru</i> – <i>U. miyabi</i>	<i>U. chanpuru</i> – <i>U. nakama</i>	<i>U. miyabi</i> – <i>U. nakama</i>
<i>External morphology</i>						
Polyp diameter	Size	Minimum	0.040*	0.683	0.016*	0.092
		Maximum	<0.001*	0.006*	<0.001*	0.269
Distance between polyps	Size	Minimum	0.013*	0.013*	0.067	0.484
		Maximum	0.006*	0.002*	0.239	0.088

* *p*-values with a significant statistical difference (<0.05).

belonging to the genus *Parazoanthus* clade “A” sensu Sinniger et al. (2010). The sequence of specimen 47J was consistently separated from *U. chanpuru* in trees of all other markers. The analyses of mt 16S-rDNA and ALG-11 nuDNA regions collapsed the sister species *U. miyabi* and *U. nakama* into a single well supported clade sister to *U. chanpuru*. The ITS-rDNA region showed the highest resolution and variability between and inside species, and clearly supported the separation of the species groups *U. chanpuru*, *U. miyabi* and *U. nakama* partially corroborating the results seen in the COI analyses, but also additionally showed six subclades with differing degrees of support inside *U. chanpuru*. Additionally, the ITS-rDNA tree strongly supported the association of the sequence from specimen 47J with sequences from *U. parasiticus* (Fig. 7), and this relationship was also weakly seen in the mt 16S-rDNA tree, pointing out the possibility of an additional undescribed Pacific sibling species to the widely known Caribbean sponge-associated *U. parasiticus*.

The mt 16S-rDNA region was very useful in distinguishing the new genus *Umimayanthus* from all the other zoantharian genera including *Parazoanthus* (clades C and A in Sinniger et al., 2010). *Umimayanthus* spp. have a unique insertion of 9 bp length (from position 556 bp to 564 bp) and one unique deletion of 14 bp length (from position 574 bp to 587 bp), both of them strongly conserved among all three new species described in this study (Fig. 2) as well as in the sequence of *U. parasiticus* (GenBank: AY995938). However, the fact that the mt 16S-rDNA tree (Fig. 9) failed to show

strong support for the association of sample 47J and *U. parasiticus* and collapsed the sister species *U. miyabi* and *U. nakama* into a single clade, calls into question the utility of mt 16S-rDNA alone as a marker to distinguish between very closely related parazoanthid species (Sinniger et al., 2010), at least inside the new genus *Umimayanthus*. The lack of resolution presented by the mt 16S-rDNA region in our results is actually consistent with results obtained by Sinniger et al. (2008), where it was not possible to distinguish between sequences of *P. axinellae* and *P. anguicomus* using this marker.

The validity of species *U. miyabi* and *U. nakama* is supported by the majority of our phylogenetic results, as well as by previous phylogenetic studies performed by Sinniger et al. (2010); in which sequences of *Umimayanthus* sp. NC Shallow1, *Umimayanthus* sp. NC Shallow2, *Umimayanthus* sp. NC Shallow3 and *Umimayanthus* sp. Japan were compared, along with other sequences. In the ITS-rDNA phylogenetic trees of Sinniger et al. (2010), these three sequences are part of a well-supported subclade inside *Parazoanthus* “clade B” (from Sinniger et al., 2010), and in our analyses of the ITS-rDNA and COI-mtDNA regions they were divided in two sister subclades. One of the subclades contained *Umimayanthus* sp. NC Shallow1, *Umimayanthus* sp. NC Shallow2 and *Umimayanthus* sp. NC Shallow3, and the second subclade included the sequence of *Umimayanthus* sp. Japan (for the equivalent abbreviations in Sinniger et al., 2010 follow GenBank accession numbers in Table B.1). These two subclades are described here as

Table 3General morphological and geographical characteristics of some sponge associated species of Parazoanthidae, including *Parazoanthus* (clades A & C) and *Uimimayanthus*.

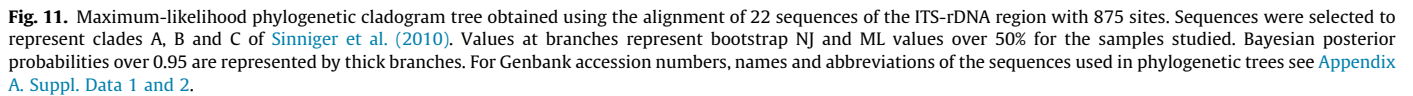
	Parazoanthidae	N. Atlantic (incl. Caribbean)	S. Atlantic	N. Pacific	S. Pacific	Mediterranean Sea	Indian (incl. Red Sea)	Artic	Antarctic	GenBankdata	Diameter (mm)	# Tentacles	References
Genus-level group	<i>Uimimayanthus</i> spp.			●	●		●			Yes	0.16–2.3	14–22	
	<i>Parazoanthus</i> spp. (Clade A)	●	●		●	●	●	●	●	Yes	2–8 (~10)	24–48	
<i>Parazoanthus</i>	Clade C	<i>Parazoanthus</i> spp. (Clade C)	●							Yes	1–1.5	20–24	
		<i>P. puertoricense</i> West, 1979	●							Yes	1.5	24	West (1979)
		<i>P. catenularis</i> (Duchassaing and Michelotti, 1860)	●							Yes	1	20–24	Duchassaing and Michelotti (1860), West (1979)
													Carlgrén (1913)
	Clade A	<i>P. anguicomus</i> (Norman, 1868)	●							Yes	4–8	36–38**	
		<i>P. haddoni</i> Carlgrén, 1913	●					●		No	10	36–46	Carlgrén (1913)
		<i>P. antarcticus</i> Carlgrén, 1927							●	No	4.5–5	32–36**	Carlgrén (1927)
		<i>P. axinellae</i> (Schmidt, 1862)	●			●				Yes	2–3.5	28–38	Herberts (1972)
		<i>P. swiftii</i> (Duchassaing and Michelotti, 1860)	●							Yes	2.5	26	West (1979)
		<i>P. capensis</i> Duerden, 1907	●	●			●			Yes	3–4	NA	Cutress (1971), Morphbank (2015)
	<i>Uimimayanthus</i>	<i>P. aruensis</i> Pax, 1911					●			No	5	36**	Pax (1911)
		<i>P. darwini</i> Reimer and Fujii, 2010				●				Yes	3–6	24–30	Reimer and Fujii (2010)
		<i>P. elongatus</i> McMurrich, 1904				●				Yes	6	40–48	Sinniger and Häussermann, 2009
		<i>P. juan-fernandezii</i> Carlgrén, 1922				●				Yes	5	40–42 (~44)	Carlgrén (1922)
		<i>P. lividum</i> Cutress, 1971*				●				No	2	29	Cutress (1971)
		<i>U. parasiticus</i> (Duchassaing and Michelotti, 1860)	●							Yes	1–1.5	28	West (1979)
		<i>U. chanpuru</i>			●	●	●			Yes	0.16–2.15	14–20	This study
		<i>U. miyabi</i>			●	●				Yes	0.29–1.70	18–22	This study
		<i>U. nakama</i>			●					Yes	0.62–2.3	20–22	This study

All distribution data was extracted from OBIS, 2015.

* This species is potentially part of genus *Uimimayanthus*, considering the characteristics giving in the original description (Cutress, 1971).

** Numbers extrapolated from the number of mesenteries.

Diameter sizes of polyps include fixed and living specimens.



U. chanpuru is consistently presented as sister to species *U. miyabi* and *U. nakama* in the phylogenetic trees of all DNA markers, and the many specimens are unified by low to medium sand content in tissues. Additionally, several statistically significant differences were found between *U. chanpuru*, *U. miyabi* and *U. nakama* with regards to cnidae sizes (Table 1 and Fig. 5) and morphology, supporting the basal separation of these main clades as seen across the different phylogenetic trees (Figs. 7–10). Between *U. chanpuru* and *U. miyabi* we found significant differences in maximum polyp sizes and distances between polyps (Table 2); in cnidae analyses significant differences were found in maximum spirocyst sizes, frequencies of b-mastigophores, and both minimum and maximum sizes of holotrichs-1 (Table 1). Between *U. chanpuru* and *U. nakama* significant differences were found in polyp size ranges (Table 2); in cnidae analyses significant differences were found in holotrichs-1's frequency, minimum and maximum sizes, and also with b-mastigophores maximum and minimum sizes (Table 1).

Our results support that *Parazoanthus* is a paraphyly consisting of at least three clades as previously mentioned (clades A, B, C in Sinniger et al., 2010). In this study we corroborate “subclade B”, as mentioned in Sinniger et al. (2010), as a valid taxonomic and phylogenetic genus-level grouping. The type species for *Parazoanthus*, *P. axinellae*, is within clade A of Sinniger et al. (2010), and in this study we split clade B from *Parazoanthus* into

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2015.04.002>.

References

- Belinky, F., Szitenberg, A., Goldfarb, I., Feldstein, T., Wörheide, G., Ilan, M., Huchon, D., 2012. ALG11-A new variable DNA marker for sponge phylogeny: comparison of phylogenetic performances with the 18S rDNA and the COI gene. *Mol. Phylogenet. Evol.* 63 (3), 702–713.
- Carlgrén, O., 1913. Zoantharia. The Danish Ingolf-Expedition 5 (4), 55–60.
- Carlgrén, O., 1922. Actiniaria und Zoantharia von Juan Fernandez und der Osterinsel. In: Skottsberg, C. (Ed.), 1880–1963. The Natural History of Juan Fernandez and Easter Island 1921–1940, pp. 148–149 (Chapter 20).
- Carlgrén, O., 1927. Actiniaria and Zoantharia. Further zoological results of the Swedish Antarctic Expedition 2(3), 1–102.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552.
- Cavalli-Sforza, L.L., Edwards, A.W.F., 1967. Phylogenetic analysis: models and estimation procedures. *Am. J. Hum. Genet.* 19, 233–257.
- Crocker, L.L., 1978. The Ecology of the Zoanthid-Sponge Symbiosis in Barbados. Montreal, Canada, Master of Science. Marine Sciences Center McGill University, pp. 23–98.
- Crocker, L.A., Reiswig, H.M., 1981. Host specificity in sponge-encrusting Zoanthidea (Anthozoa: Zoantharia) of Barbados, West Indies. *Mar. Biol.* 65, 231–236.
- Cutress, C.E., 1971. Corallimorpharia, Actiniaria and Zoanthidea. In: Edmund, D.G. (Ed.), *Memoirs of the National Museum of Victoria* 32, Australia, pp. 89–90 (Chapter 7).
- Delage, Y., Hérouard, E., 1901. Zoanthidés. – Zoanthidae. In: *Traité de Zoologie concrète*, Tome II – 2e Partie. Les Coelentérés. C. Reinwald, Paris, 848pp.
- Duchassaing, P., de F., Michelotti, J., 1860. Mémoire sur les Coralliaires des Antilles. Imprimerie Royale, Turin, 112pp., pls. 1–11. [Note: This is separate of a publication with identical title and text in the *Memorie della Reale Accademia delle Scienze di Torino*, ser. 2, vol. 19, pp. 279–365, that appeared in 1861].
- England, K.W., 1991. Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. *Hydrobiologia* 216 (217), 691–697.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.* 3, 294–299.
- Fujii, T., Reimer, J.D., 2011. Phylogeny of the highly divergent zoanthid family Microzoanthidae (Anthozoa, Hexacorallia) from the Pacific. *Zool. Scr.* 40, 418–431.
- Griekspoor, A., Groothuis, T., 2005. 4Peaks, Version 1.6. Available from <<http://www.mekentosj.com/>>.
- Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52 (5), 696–704.
- Haddon, A.C., Shackleton, A.M., 1891a. The Zoanthaeae. In: A revision of the British Actiniae. Part II. Reports on the zoological collections made in the Torres Straits by A.C. Haddon, 1888–1889. *Scientific Transactions of the Royal Dublin Society*, ser. 2, 4(12), 609–672, pls. 58–60. [Published November 1891].
- Haddon, A.C., Shackleton, A.M., 1891b. Actiniae: I. Zoanthaeae. In: Reports on the Zoological Collections Made in the Torres Straits by Professor A.C. Haddon, 1888–1889. *Scientific Transactions of the Royal Dublin Society*, ser. 2, 4(13), 673–658, pls. 61–64. [Published December 1891].
- Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22, 160–174.
- Herberts, C., 1972. Etude systématique de quelques zoanthaires tempérés et tropicaux. *Tethys Suppl.* 3, 69–156.
- Hidaka, M., 1992. Use of nematocyst morphology for taxonomy of some related species of scleractinian corals. *Galaxea* 11, 21–28.
- Hidaka, M., Miyazaki, I., Yamazato, K., 1987. Nematocysts characteristic of the sweeper tentacles of the coral *Galaxea fascicularis* (Linnaeus). *Galaxea* 6, 195–207.
- Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23 (21), 2947–2948.
- Montenegro, G.J., Acosta, A., 2010. Habitat preference of Zoantharia genera depends on host sponge morphology. *Universitas Scientiarum* 15 (2), 110–121.
- Morphbank, 2015. Biological Imaging. Available at <<http://www.morphbank.net/>> (accessed 13.03.15).
- OBIS, 2015. Ocean Biogeographic information System. Available at <<http://iobis.org/mapper/>> (accessed 13.03.15).
- Pax, F., 1911. Aktinien der Aru-Inseln. pp. 300–302. In: *Senckenbergische Naturforschende Gesellschaft*. 1911. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, vol. 33 (1910–1911). Waldemar Kramer, 592pp.
- Rambaut, A., 2002. Se-Al: Sequence Alignment Editor, Version 2.0a11. Available at <<http://tree.bio.ed.ac.uk/software/seal/>>.
- Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *J. Mol. Evol.* 43, 304–311.
- Rasband, W.S., 2012. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <<http://imagej.nih.gov/ij/>>, 1997–2012.
- Reimer, J.D., Fujii, T., 2010. Four new species and one new genus of zoanthids (Cnidaria, Hexacorallia) from the Galápagos Islands. *ZooKeys* 42, 1–36.
- Reimer, J.D., Sinniger, F., 2010. Unexpected diversity in Canadian Pacific zoanthids (Cnidaria: Anthozoa: Hexacorallia): a molecular examination and description of a new species from the waters of British Columbia. *Mar. Biodiversity* 40 (4), 249–260.
- Reimer, J.D., Sinniger, F., Fujiwara, Y., Hirano, S., Maruyama, T., 2007a. Morphological and molecular characterisation of *Abyssosanthus nankaiensis*, a new family, new genus and new species of deep-sea zoanthid (Anthozoa: Hexacorallia: Zoantharia) from a north-west Pacific methane cold seep. *Invertebrate Syst.* 21, 255–262.
- Reimer, J.D., Takishita, K., Ono, S., Tsukahara, J., Maruyama, T., 2007b. Molecular evidence suggesting intraspecific hybridization in *Zoanthus* (Anthozoa: Hexacorallia). *Zool. Sci.* 24, 346–359.
- Reimer, J.D., Sinniger, F., Hickman Jr., C.P., 2008. Zoanthid diversity (Anthozoa: Hexacorallia) in the Galapagos Islands: a molecular examination. *Coral Reefs* 27, 641–654.
- Reimer, J.D., Nakachi, S., Hirose, M., Hirose, E., Hashiguchi, S., 2010. Using hydrofluoric acid for morphological investigations of zoanthids (Cnidaria: Anthozoa): a critical assessment of methodology and necessity. *Mar. Biotechnol.* 12 (5), 605–617.
- Reimer, J.D., Lorion, J., Irei, Y., Hoeksema, B.W., Wirtz, P., 2014a. Ascension Island shallow-water Zoantharia (Hexacorallia: Cnidaria) and their zooxanthellae (*Symbiodinium*). *J. Mar. Biol. Assoc. United Kingdom*. <http://dx.doi.org/10.1017/S0025315414000654>.
- Reimer, J.D., Poliseno, A., Hoeksema, B.W., 2014b. Shallow-water zoantharians (Cnidaria, Hexacorallia) from the Central Indo-Pacific. *ZooKeys* 444, 1.
- Reimer, J.D., Uyeno, D., Berumen, M.L., 2014c. First records of Parazoanthidae and Microzoanthidae (Anthozoa: Hexacorallia: Zoantharia) from the Red Sea. *Mar. Biodiversity Rec.* 7, e7.
- Rodriguez, F., Oliver, J.L., Marin, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* 142, 485–501.
- Rützler, K., 2002. Family Spirastrellidae Ridley & Dendy, 1886. In: John, N.A.H., Rob W.M.V. (Eds.), *Systema Porifera*. Springer, US, pp. 220–223.
- Ryland, J.S., 1997. Reproduction in Zoanthidea (Anthozoa: Hexacorallia). *Invertebrate Reprod. Dev.* 31, 177–188.
- Ryland, J.S., Lancaster, J.E., 2003. Revision of methods for separating species of *Protopolythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. *Invertebrate Syst.* 17, 407–428.
- Ryland, J.S., de Putron, S., Scheltema, R.S., Chimonides, P.J., Zhadan, D.G., 2000. Semper's (zoanthid) larvae: pelagic life, parentage and other problems. *Hydrobiologia* 440, 191–198.
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.
- Schmidt, H., 1974. On evolution in the Anthozoa. In: *Proceedings of the 2nd International Coral Reef Symposium* 1, pp. 533–560.
- Sinniger, F., 2007. Zoantharia of New Caledonia. In: Payri, C.E., Richer de Forges, B. (Eds.), *Compendium of Marine Species from New Caledonia: second edition*. Doc. Sci. Tech. I17, Second edition, France, Nouméa IRD, 435p. +39p, 2–3.
- Sinniger, F., Häussermann, V., 2009. Zoanthids (Cnidaria: Hexacorallia: Zoantharia) from shallow waters of the southern Chilean fjord region with the description of a new genus and two new species. *Organ. Diversity Evol.* 9, 23–36.
- Sinniger, F., Montoya-Burgos, J.L., Cheveldonné, P., Pawlowski, J., 2005. Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial ribosomal genes. *Mar. Biol.* 147, 1121–1128.
- Sinniger, F., Reimer, J.D., Pawlowski, J., 2008. Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia). *Zool. Sci.* 25, 1253–1260.
- Sinniger, F., Reimer, J.D., Pawlowski, J., 2010. The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: description of two new genera. *Mar. Biodiversity* 40, 57–70.
- Sinniger, F., Ocana, O.V., Baco, A.R., 2013. Diversity of zoanthids (Anthozoa: Hexacorallia) on Hawaiian seamounts: description of the Hawaiian gold coral and additional zoanthids. *PLoS one* 8 (1), e52607.
- Swain, T.D., 2009. Phylogeny-based species delimitations and the evolution of host associations in symbiotic zoanthids (Anthozoa, Zoanthidea) of the wider Caribbean region. *Zool. J. Linn. Soc.* 156, 223–238.
- Swain, T.D., 2010. Evolutionary transitions in symbioses: dramatic reductions in bathymetric and geographic ranges of Zoanthidea coincide with loss of symbioses with invertebrates. *Mol. Ecol.* 19, 2587–2598.
- Swain, T.D., Wulff, J.L., 2007. Diversity and specificity of Caribbean sponge–zoanthid symbioses: a foundation for understanding the adaptive significance of symbioses and generating hypotheses about higher-order systematics. *Biol. J. Linn. Soc.* 92, 695–711.
- West, D.A., 1979. Symbiotic zoanthids (Anthozoa: Cnidaria) of Puerto Rico. *Bull. Mar. Sci.* 29 (2), 253–271.
- WoRMS, 2015. World Register of Marine Species. Available at <<http://www.marinespecies.org/>> (accessed 24.03.15).